

Should Reproductive Biology be Encouraged?

FERTILIZING human eggs in the test tube and using prostaglandins as an abortion pill are spectacular ideas which have captured attention recently. But in the past year or so there have been advances in almost all aspects of human reproductive biology, although this work has been more in the nature of pinpointing the areas which can most profitably be exploited than in providing any definite answers. In this context, it seems likely that virtually all scientists will regard as timely the plans of the World Health Organization to inject considerable funds into some aspects of research on human reproduction. (An account of the programme will be found in *Nature New Biology* next Wednesday.)

Reproductive biology is, inevitably, a controversial subject and the promise to some, a threat to others, that research will make a revolution in social as well as in scientific thinking is reflected in last week's attacks by two Nobel Laureates, Dr James Watson and Dr Max Perutz, on the work of Dr R. G. Edwards and his colleagues from Cambridge. There is every prospect that, if successful, Edwards's work will lead to the development of procedures to allow human eggs to be fertilized *in vitro* and the embryos then to be reimplanted at an early stage of their growth. But although this technique may be able to overcome some forms of infertility, Watson and Perutz argue that it involves very great and quite unjustified risks of producing a defective embryo.

But whether or not what seems likely to develop into a campaign to stop such work is successful, other changes in reproductive biology are almost certainly in train. It will not be surprising, for example, if recent developments in distinguishing sperm carrying a Y-chromosome from those bearing an X-chromosome should lead to a ready technique for separating sperm according to whether they determine a male or female child. Coupled with the use of artificial insemination this finding implies that it may become possible to choose the sex of progeny.

Not everyone will agree on the value of advances of this nature, but research on embryogenesis itself has in general produced results which can universally be applauded. Immunological studies of foeto-maternal relationships, for example, have already proved their use in helping rhesus-positive babies to survive in rhesus-negative mothers. Indeed, histo-incompatibility studies on foetal development may lead to a better understanding of the processes which may go wrong (of which there seem to be many) in earlier embryogenesis. Studies on the presence of antigens on sperm may also be fruitful.

Spermatogenesis and the ovulatory cycle are ill-defined by comparison and it is this area of research which will benefit most from the expanded programme by which WHO hopes to increase understanding of the processes of human reproduction. The aim of the programme is to develop safe and effective methods for regulating human fertility. Research is planned on a broad basis. One reason for this is that WHO must take account of social mores which may mean that particular methods of contraception may not be acceptable in some countries.

Another is that it is at present difficult to decide just what will prove to be the best ways of improving physiological contraception.

The decision is a sensible one to concentrate research on the basic processes which lead to sperm formation, maturation and transport and on the release, fertilization, implantation and early development of the egg. Even the stages of the ovulatory cycle are not at present known in much detail and just a slight increase in knowledge could cast a valuable light, for example, on what are the dangers of the rhythm method of contraception. A large part of the research will concentrate on the biochemistry of steroid action on tissues of the female reproductive system which certainly seems to offer the best prospects for improving contraception in the short term; fears about present contraceptive pills may be eased and better ones may be developed. The programme is also strong in that there will be centres which will conduct clinical trials of any possible fertility regulating agents.

It is all to the good that WHO seems to have decided that it is not sufficient just to conduct research and then to let the results look after themselves. An integral part of the programme is to help disseminate to other scientists the results which emerge from research on reproductive biology. There seems to be less certainty over just how this is to be achieved than there is about the research to be promoted but scientists will hope that a decision is taken and put into effect soon; plans for WHO to establish a Documentation Centre will no doubt be watched as an example of how communication can be improved in specific research areas.

How can WHO best support this ambitious programme of research? About \$500,000 per year is at present spent by WHO on research in various aspects of human reproduction and this programme will continue. The new programme, however, with a projected income for its first year of nearly \$7 million is likely to have more impact. Funds for the programme are being gathered from many sources and scientists will be anxious to see how well the plans work to organize research on rather closely defined lines.

Because of its international nature, particular problems must face WHO in organizing this programme. One of WHO's weaknesses is at the same time a strength; to be successful, the programme must take into account the susceptibilities of the member states of WHO in general and the funding agencies in particular. This means that justice must be seen to be done in apportioning funds to research institutions in different countries. The first consideration of the programme must, of course, be to ensure that the research supported is of proper quality. But it is no use providing answers if no one is willing to listen to them. The strength of WHO's approach is that any successes of a programme organized on such a wide basis will be likely to have an equally wide influence. Those who would judge the programme, therefore, should be duly sympathetic if at times it seems that it will take longer than it might to come up with the answers.



Poor Report for the Post Office

POSTS and telecommunications are no more suited to being part of the same enterprise than are ocean liners and aircraft. Yet history has lumped them together and organizations like the British Post Office must make the best of it. The best is none too good, as the report of the Post Office's first full year as a public corporation shows (*Post Office Report and Accounts, 1970-71*, HMSO, £1.50). So great were the losses on the postal services that the astonishing profits of £93.5 million on the telecommunications side left the corporation with a total profit of only £20.5 million, the lowest turned in since 1964-65.

To be sure, the seven-week strike of early 1971 hurt—all considered, it cost the corporation about £13 million—but the bulk of the postal loss of nearly £73 million was owed simply to the facts that it costs a great deal of money to hire people to carry messages by hand and that the public demands that its messages be carried cheaply. Mr Bill Ryland, the Post Office's new chairman, has said that increased postal charges are inevitable and the choice must be made between substantial price increases or lower quality service. He refused to be pessimistic about the future of the postal services while taking comfort from the health of the telecommunications service. But it is hard to share his pleasure in the telecommunications performance. What is so depressing about the corporation's first full report is the utter lack of promise of a sophisticated telephone system for Britain in the near future. While the vast profits from telecommunications are a great solace to the Post Office, they have not done much for the telephone subscriber. In the past year, the waiting list for new telephones has grown from 108,000 to 121,000, while the quality of the subscriber trunk dialling (STD) service deteriorated. The Post Office figures, always more conservative than those of the ordinary frustrated telephone user, concede that 8.7 per cent of calls failed because of faults in the system and calls made through the operator were also slightly more troublesome. In 1970-71 during the day it took the operator nearly 6.5 seconds to answer—the poorest record since 1966-67.

The Post Office admittedly is embarked on an extensive programme to expand and modernize the telephone service, and it is now spending about £500 million a year to make up for the mistakes—the Treasury's as well as its own—of the 1950s and early 1960s. Yet the report does not have cheerful things to say about the progress in modernizing telephone exchanges which are the bottleneck to any drastic improvements of the present system. Because so much existing equipment is of the old fashioned step-by-step Strowger variety, the Post Office had to spend £5.5 million in 1970-71 to buy more of the same, to keep the system running. Moreover, its independence as a corporation has not helped it find a solution to its chronic problem of the late delivery of equipment by the British telecommunications industry. In fact, in the past year, fewer orders were filled than the year before, and the average delay on exchange contracts was thirteen months, with the newer, more desirable crossbar exchange equipment even further behind schedule than the Strowger.

Perhaps the most worrying statements of the Post Office's report are "the relative cheapness of the telephone

is creating a very heavy demand which is increasingly difficult to meet with present resources." "Waiting lists are bound to increase and service deteriorate until prices can be adjusted." What irks the Post Office is that while telephone calls are profitable, the average return on the rental of a telephone set is not. To put this right it would like to raise the rental—and drive some of those prospective customers away. The Post Office has not yet learned to concentrate on the golden eggs and forget the cost of buying the geese.

It is worrying that the Post Office misinterprets the demand for telephones. The waiting list refuses to diminish—not because telephone rentals are cheap but because the public is recognizing that telephone calls are cheaper and often superior (because they are instantaneous and two-way) as a form of communication than the letter. This recognition could be one of the Post Office's most valuable assets, if it chose to make it so. Because it is saddled with running both a postal service and a telephone system, why does it not argue publicly the advantages of using one over the other? British telephones are under-used and telephone calls on which the Post Office made so much profit last year were made from instruments which are used, on the national average, only twice a day. For that matter, the Post Office should be putting more effort into making cheap, throw-away telephones, and at least, insisting that telephone wires and outlets be installed in all new buildings.

The most essential task for the Post Office is to recognize that it is in the telephone service, not the moribund post, where the real emergency lies.

100 Years Ago



THE Inaugural Meeting of the Newcastle College of Physical Science on Tuesday last week was a great success. The Council decided unanimously, on the application of upwards of seventy ladies, to make no distinction of sex in the admission of pupils, placing all on a footing of exact equality. The total number of students admitted up to the time of the inaugural ceremonial, was fifty-one. In contrast to this we may note that last week the governing body of the University of Edinburgh rejected by a small majority Dr. Alexander Wood's motion, "That, in the opinion of this Council, the University authorities have, by published resolutions, induced women to commence the study of medicine at the University; that these women, having prosecuted their studies to a certain length, are prevented from completing them for want of adequate provision being made for their instruction; that this Council, without again producing any opinion on the advisability of women studying medicine, do represent to the University Court, that, after what the Senatus and Court have already done, they are at least bound in honour and justice to render it possible for those women who have already commenced their studies to complete them."

From *Nature*, 5, 13, November 2, 1871

OLD WORLD

RESEARCH AND DEVELOPMENT

Constructive Advice

THE Institution of Professional Civil Servants feels that the British government should provide increased support for its industrial establishments so that Britain can compete on equal terms with other European countries. This advice is given by the IPCS to the Department of Trade and Industry as a contribution to the DTI's review of industrial research establishments (*The Role of the Government's Industrial Research Establishments in the 1970s*, IPCS).

The IPCS stresses that the advantages of such reviews are nullified if they are held too often and the institution feels that this has been the case recently—several establishments are now being reviewed for the third time in ten years. This almost continuous series of reviews coupled with changes that have placed some establishments under the aegis of four different departments in the same period has, in the view of the IPCS, led to uncertainty about jobs and prospects and a loss of morale.

The establishments now under review are the Laboratory of the Government Chemist, the National Engineering Laboratory, the National Physical Laboratory, the Safety in Mines Research Establishment and the Warren Springs Laboratory together with the United Kingdom Atomic Energy Authority establishments at Harwell and Risley. The IPCS sees a scope for increasing the work of these establishments, in particular by an increase in their contract work for individual companies. For this to be achieved, however, it will be necessary to increase the powers of the directors of the establishments so that they can exercise commercial judgment and develop the work as they see fit.

In his introduction to the report, Mr William McCall, general secretary of the IPCS, is possibly previewing Lord Rothschild's review of civil science when he calls for public research and development establishments to contribute more to the industrial and economic well-being of Britain. He also re-states the institution's view that these establishments should not diversify but should continue their work in basic long-term research.

The report comes out strongly against the idea that these establishments should pay their own way and it criticizes modern innovation theory, which holds that all industrial research should be self-sufficient. To substantiate this position Bell Telephone Laboratories Inc. is cited as a large company that has failed to keep its

research and development self-sufficient. A plea is also made for more research and development resources to be made available to industry to supplement its own efforts and the IPCS points out that the industrial research establishments could well fit this bill.

The central theme of the report is that the present support of research and development by the government is biased towards defence, aerospace, electronics and nuclear power whereas industries that contribute most to the balance of payments—the mechanical and engineering industries for example—have been sadly neglected. The institution says that this imbalance should be corrected by providing more support for these industries at government establishments, provided however, that this is accompanied by a corresponding increase in research and development effort within the industry itself.

Perhaps the most important plea made by the IPCS is for increased support for industrial research and development so that Britain will not be handicapped in competition with other countries, in particular members of the European Economic Community. While emphasizing that support should not be increased merely because other countries are supporting both civil and military technology to a greater degree than Britain, the institution feels that British industry would be severely handicapped if there were no increase in support for industrial research and development.

EDUCATION

A-level Broadening

MORE schoolchildren are opting for A-level courses involving both science and other subjects, according to a report of the Department of Education and Science (*Statistics of Education*, 1, HMSO; £1.70). At the beginning of 1970 31.0 per cent of the 254,000 A-level pupils in schools in England and Wales were following courses comprising science subjects alone and a further 50.9 per cent were studying non-scientific courses; 18.1 per cent of the courses, however, contained both science and non-science subjects. In January 1969, the proportions were 31.0 per cent, 52.1 per cent and 16.9 per cent respectively.

During 1969 the total number of pupils of all ages rose by 2.4 per cent to 8.6 million, of which about 8 million were in maintained schools. The total teaching strength increased by 3.3 per cent and the pupil-teacher ratio dropped from 27.7 to 27.4 in maintained primary schools and from 17.9 to 17.8 in maintained secondary

schools while in direct grant schools the ratio is 15.5.

The proportion of 15-year-old children continuing their school careers still shows large regional variations, but the figure for the north region, 46.9 per cent on January 1, 1970, compared with 44.9 per cent a year before, reflects a larger increase than that for the south-east region (63.8 per cent compared with 62.5 per cent). In 1966, 53.2 per cent of 15-year-old pupils in the south-east region stayed on at school beyond the statutory leaving age.

Reliving the Past

OVERHEARD at a reception at 21 Albemarle Street, London, last week: "I've not visited this place before; it's rather marvellous, isn't it?" Not such an extraordinary remark in the normal course of talk at a social gathering, but the speaker and the occasion gave it special meaning. With true British feeling for the past, the 172-year-old Royal Institution of Great Britain stepped back in history on October 20 and for three hours became once more the assembly rooms of London society. It had thought to ask to a party (no doubt with an eye, somewhat disguised, on some generous benefactions) as many descendants as could be traced of its founders, early proprietors and officers to meet some of the present members, to hear something of its history and functions today and to see its collection of archives and historic scientific apparatus.

Because so many distinguished and wealthy people directly associated themselves with the Royal Institution in its early days and supported it with subscriptions, it is not surprising that the list of descendants at the party read like an extract from *Debrett's Peerage* (although, of course, it is just these people who can most easily be traced). Few of the guests were practising scientists, although among them was the engineer Sir Richard Young, a descendant of Thomas Young who was one of the institution's first professors. The Percy family have long had an honourable connexion with the Royal Institution—the fourth, sixth, seventh and eighth Dukes of Northumberland were all presidents as was Lord Eustace Percy—and it was therefore fitting that the guest of honour was the tenth Duke of Northumberland, at present chairman of the Medical Research Council.

RESEARCH AND DEVELOPMENT

Summer Lightning

RESEARCH has begun into the possibility of providing Britain with an electricity transmission system of up to one and a half million volts. Since August, scientists at the Central Electricity Research Laboratories (CERL), using a gantry forty metres high and a Ferranti impulse generator capable of an open circuit output of 5.2 million volts, have been working on the basic problems of such a system.

The CEBG's new 400 kV supergrid is not yet fully commissioned but CERL has started work on ultra high voltage (uhv) because the time required to make a system operational means that research must be many years ahead of possible application. So far, each time a higher voltage network has been added, the voltage has roughly doubled—from 132 to 275 kV in 1950 and from 275 to 400 kV in 1960—but it has been suggested that the next step ought to be a trebling rather than a doubling of present levels—to 1,300 kV rather than 800 kV—because of the capital cost involved. It is also unlikely that demand will exceed the capacity of a 1,300 kV network in the foreseeable future.

Work on the basic data for an 800 kV system has already been completed and CERL is at present trying to find insulators capable of resisting lightning strikes and the surges resulting from switching in a uhv system. With higher voltages there are ever increasing difficulties with insulation and it has been suggested that uhv transmission will

prove impossible; at CERL, however, they are confident it can be done and hopeful that it will prove possible to carry uhv on a single circuit eight conductor bundle on towers no higher than those used at present.

The porcelain insulators at present under test have withstood simulated lightning strikes of up to 5 million volts without breakdown but switching surges, which can be expected to reach two and a half times the nominal rating of a transmission system, are tending to break down at about 2.25 million volts.

The facilities, sited at Leatherhead on the edge of the Surrey green belt, are the world's first completely all-weather uhv test facilities; they will be used in close cooperation with the CEBG's Brighton site where work will shortly begin on the other main problem of transmission, the reduction in insulation efficiency due to pollution. Salt in the atmosphere is the worst enemy, and the Brighton site, right on the coast, is ideal for studying this.

The capital cost of the Leatherhead and Brighton sites is about £500,000. Cost was one of the two main factors that resulted in outdoor sites—to put only the Leatherhead facilities indoors would have cost another £2 million. The other factor was the firm belief of Dr John Forrest, director of CERL, that a uhv grid will finally have to work outdoors so it might as well start that way. The power frequency tests, which need to be done indoors, can be accommodated later in the existing laboratories.

It is in fact doubtful if a grid of more than a million volts will ever be built. If the predicted 5.5 per cent per annum load growth rate for the later 1970s continues, then the present 400 kV network will be good for the next 20 years. Whether a 1,300 kV network (the likeliest voltage to be adopted over one million volts) will then be built will depend on the nature of power transmission by the 1990s; centralization resulting in fewer, bigger generating plants might well demand it, but a change in public attitudes to the siting of nuclear power stations near large towns, for example, would make it unnecessary. Whatever happens, uhv will not replace but only supplement the 400 kV network, and it is likely to be used only for the large-scale, long-distance transmissions from, say, the Midlands to London.

SOVIET SCIENCE

Industrial Research

THE recent "Scientists for Industry" seminar held in Rostov-on-Don on October 13–15, 1971, has again brought to the fore the apparent lack of contact between research and development and industrial application in the Soviet Union. The Rostov seminar was devoted to problems of expanding research in technical colleges, in accordance with the dictates of industry, but background material published in the Russian press suggests the existence of a more urgent problem than was dealt with at the Rostov meeting.

Writing in Pravda (No. 278, 1971), Academician Aleksandr Ishlinskii, chairman of the All-Union Council of the Scientific-Technical Society of the USSR, stresses the need for the development of research and development laboratories within factories that could provide one of the chief links in technological progress. Unfortunately, in many establishments, they are poorly equipped outbuildings, unsuitable for serious research and many factory directors consider them to be second-class departments. Although specialists working in these laboratories are entitled to a similar salary to that of scientists in pure research institutes, very few directors are willing to implement such payments. Accordingly, there is a serious leakage of scientists from industry, particularly in the highly qualified classes.

A closer cooperation between science and industry was one of the principal resolutions of the Twenty-Fourth Party Congress held earlier this year, and the factory laboratory has often been commended as an excellent means of implementing this cooperation. But, without more attention and funds, they may act as a deterrent to cooperation.



The CERL facilities at Leatherhead showing the Ferranti 5.2 million volt impulse generator and the test gantry that permits possible tower designs to be examined, and substation plant to be tested alongside. Eight conductors in a metre bundle are suspended from the gantry on the porcelain insulator strings.

SCIENTIFIC INFORMATION

One Step Nearer

FIRST plans to help keep scientists and engineers in touch on a world-wide basis with the latest published work in their field have been approved at a UNESCO conference.

At a meeting in Paris earlier this month delegates from eighty-three countries and thirty-nine international organizations approved the recommendations made in October of last year to establish a World Science Information System, known as UNISIST (see *Nature*, **230**, 544; 1971).

The intention behind UNISIST is that it will help overcome the "information explosion" which has resulted in about two million scientific and technical articles being published each year in 70,000 specialized journals; it will also make the work already published more easily available. UNISIST is the joint brainchild of UNESCO and the International Council of Scientific Unions, and its protagonists claim that its advantages are, first, that it is a world-wide organization that involves both governments and scientific organizations and, second, that unlike earlier plans for world-wide systems it is comparatively flexible, coordinating existing networks rather than establishing a new central organization. The chief problems that UNISIST faces are how to interconnect existing systems of information retrieval, whether conventional or computerized, and how to solve the problems of classification.

The recommendations that the conference discussed—and passed with only minor modifications—were the work of the UNESCO-ICSU committee that was created in January 1967, and final approval of the scheme now rests with the UNESCO general conference which meets in the autumn of 1972.

After the conference the chairman, Dr Harrison Brown of the United States National Academy of Sciences, said that there was "strong east-west cooperation" in the preparation for UNISIST. The French government is to establish a centre for an International Serials Data System, and Poland is providing an international training centre to help meet the shortage of information specialists. At present UNISIST is confined to science and technology, but it is hoped to extend it to cover the social sciences and humanities if the system proves successful.

A recommendation that allowance be made for UNISIST in the 1973–74 budget of UNESCO was passed to the director general, and although no figure was named, £750,000 to £1 million as recommended in October 1970, is thought to be a likely figure.

Whether UNISIST will ever function efficiently remains to be seen and doubts have been expressed as to whether UNESCO is the best body to run such an organization. The problem of accommodating the views and interests of all the countries involved is tending to produce more talk than action, and it has been suggested that intensification of the interchange of information between existing organizations might in the end prove more effective than the more ambitious UNESCO plan.

ARCHAEOLOGY

Old Boats, New Ideas

from a Correspondent

BOATS excavated in Britain and dating from before the Norman conquest were discussed at an international symposium held earlier this month at the National Maritime Museum, Greenwich. The Ferriby boats (1600 BC to 800 BC) are the oldest yet found in northern Europe and were described by their excavator, Mr Edward Wright, who admitted that, apart from the last of the three excavations in 1963, the rescues had been archaeological disasters. The exception, Ferriby 3, is still being treated at Hull museum.

Although the ages of the Ferriby boats are quite different, they were found within a hundred yards of each other in the Humber foreshore and have unique common structural features. They are flat-bottomed with a hinged plank structure stiffened by poles pushed through cleats. Mr Wright suggested that these boats were relics from a prehistoric boatyard that had been located at the same site for centuries.

The rescue of the Ferriby boats has proved to be a cautionary tale. This time last year another ancient boat rescue was attempted, but under very different circumstances from Mr Wright's pioneering efforts. The pre-conquest Graveney boat was raised in seventy-three parts from a Kent marsh by a joint task force from the National Maritime Museum and the British Museum, and has since been the subject of a meticulous scientific examination.

The wealth of information now emerging from this painstaking work was sketched by the archaeological director of the project, Valerie Fenwick of the British Museum. Much is being learnt about trade contacts with Europe in the ninth century AD, the importance of the sea route as a highway between London and Canterbury, the relevance of the local saltworks to the battering which the boat received and so on. Several intriguing constructional details have also been deciphered; it seems, for

example, that the boat was built right side up in the open. Such information could not have been obtained without the most exhaustive study of the boat's timbers, involving the drawing of every mark on both sides of each fragment—a nine-month task.

One question raised at the symposium was how the Greenwich unit should deploy its still slim resources (see *Nature*, **233**, 226; 1971). Should it, as proposed by Mr Wright, deliberately excavate selected sites such as the sixth century BC Brigg raft still buried in a Lincolnshire brickyard or should it prepare itself for the next rescue? Ancient boats often emerge during land developments in Britain and, typically, have to be abandoned for lack of recording power and conservation facilities. Within weeks of the Graveney operation, for example, two boats of almost equal interest were found during cofferdam works in the Thames near the Mermaid Theatre. Although part of one of them was recovered, much valuable information was lost.

MUSEUMS

Keeping in Touch

HISTORY that is barely history is displayed in the Science Museum's new telecommunications gallery opened this week by Christopher Chataway, Minister for Posts and Telecommunications. The new gallery covers the development of electrical communication from the early nineteenth century to the days of colour television and a glimpse into the future is also shown.

The gallery, with an area of 10,000 square feet, provides about twice the area previously available for this collection, and is laid out on either side of a central avenue with the side bays going into particular aspects of the subject in greater detail. The north side of the gallery deals largely with line communications, the south side with radio, television and radar.

Among the more interesting exhibits are Cooke and Wheatstone's needle telegraph instruments, examples of the first transatlantic cables, and the equipment used in the Daventry test to prove the feasibility of radar in 1935, which is accompanied by a tape recording of an account of the experiment by A. F. Wilkins, who assisted Watson-Watts with the test. What is believed to be the only surviving example of the world's first centimetric radar equipment is included in the exhibition, as is the first commercially marketed video recorder.

Among the recent developments on show are a video cassette and a brief description of the possibilities of wave guides and optical fibres for carrying telecommunications signals.

NEW WORLD

New AEC Broom Begins to Sweep

by our Washington Correspondent

THE nuclear power industry is by now used to accusations that it is abusing the natural environment and endangering the health of countless Americans. Mere accusations that its quality control and safety standards are not all they might be would, in normal circumstances, scarcely merit a single raised eyebrow, but last week these accusations caused considerable consternation in the nuclear industry, for they were made by James R. Schlesinger, the new chairman of the Atomic Energy Commission. And, to rub salt in the industry's wounds, Schlesinger added that the nuclear companies need not look to the AEC to fight their battles, for the commission's most important job will be to protect the interests of the public.

These remarks were made not to an appreciative audience of environmentalists, but to representatives of the nuclear industry itself who listened with growing alarm to what Dr Schlesinger had to say in his first public speech. The remarks have been widely interpreted as marking a fundamental shift in the AEC's policies, but what they mean in effect is that the Atomic Energy Commission is fully prepared to stand by the new regulatory procedures set up in response to the appeals court decision on the Calvert Cliffs reactor (see *Nature*, 233, 85; 1971). In a sense, Dr Schlesinger will be seeking during the next few years to build up public confidence in the AEC's power to regulate the nuclear industry, and in so doing, he is likely to help the cause of nuclear power much more effectively than by siding with the nuclear industry.

The burden of Dr Schlesinger's message was that the nuclear industry has now grown past the stage at which it needs to be nurtured by the AEC, and that it ought now to be capable of standing on its own feet. "From its inception, the Atomic Energy Commission has fostered and protected the nuclear industry," he said. "Looking back, one can, I think, say that this was the right policy for that historical epoch . . . but that industry should now be on a self-sustaining basis. Those of you who regard the response to Calvert Cliffs as indicating a climatic change in the relationship between the industry and the AEC could well be right, though perhaps for the wrong reason. The move toward greater self-reliance for the

industry had a certain historic inevitability."

The AEC has been consistently criticized in the past for allowing nuclear power companies to get away with substandard engineering and in consequence the purchasing utilities have bought plants which are unduly susceptible to breakdown and poor performance. But in future, if Dr Schlesinger lives up to the promises made in his speech, the AEC will tighten its control considerably over important but unglamorous engineering details. "The AEC's role is . . . primarily to serve the public interest," Dr Schlesinger told his audience, and "those engineering details are the heart of the problem". The AEC will need to be assured that piping is of the highest quality, that pumps work and that valves are properly designed. "Those in industry who have chided us—quite properly—about the AEC regulatory process know full well that you have reason to blush regarding some of these aspects of quality assurance," Dr Schlesinger told his audience.

But the AEC will not expect to fight all the battles for the purchasing utilities for ever, for Dr Schlesinger told them they should acquire the technical expertise and trained personnel to make sure that they receive good value for their money from the builders of power plants. If the utilities acquire this expertise, it will help "to avoid power disruptions, to insure that plants can and will be properly maintained and, among other things, to avoid relying on the Atomic Energy Commission to perform this critical task".

Much of what Dr Schlesinger had to say was a bitter pill for his audience to swallow, and the nuclear power industry could perhaps be excused for choking on some of his suggestions, if only because they are reminiscent of many of the charges levelled at the industry by some of its more vocal opponents. Indeed, Dr Schlesinger had more than a good word to say for the environmentalists who have pitted their armour against the industry in the past. "A number have bad manners," he said, "but I believe that broadside diatribes against environmentalists to be not only in bad taste but wrong. I believe that we shall receive from responsible environmentalists considerable assistance in resolving our present diffi-

culties." Dr Schlesinger refused even to be drawn into the argument over whether or not society ought to curb its appetite for nuclear power, saying that "it would be inappropriate for the Atomic Energy Commission to take a position on this issue".

In a sense, the AEC has already demonstrated to the nuclear industry its new role, for in spite of great industrial pressure to appeal against the Calvert Cliffs decision, the AEC chose instead to amend its regulations. Dr Schlesinger pointed out that the new regulations present no insuperable difficulties, and "while they are tough, they are workable". He chided the nuclear industry for advising the AEC to evade the court mandate, saying "it is not the AEC's responsibility to ignore on your behalf an indication of Congressional intent". There is a clear option open to the industry if it believes the new regulations to be too harsh, and that is through the courts. There will evidently be nothing to be gained by complaining to the AEC itself.

Finally, Dr Schlesinger set about dismantling a popular misconception. "It is not the responsibility of the AEC to supply power," he said, and he went on to infer that those who have criticized the AEC for fostering the demand for electricity would do better levelling their complaints at the utilities themselves. "The AEC does not sell power reactors. We are a bystander, sympathetic I trust. The selling of power reactors is a concern of the vendors: the decision to buy that of the utilities."

Set against remarks made by Dr Glenn Seaborg, Dr Schlesinger's predecessor as chairman of the AEC, Dr Schlesinger's speech marks a change of attitude, if not of policy. In a speech to the International Platform Association, in July, for example, Dr Seaborg had little hesitation in jumping into the debate about society's need for power, and he spent much of his speech extolling the virtues of nuclear power as opposed to other forms of energy.

Why did Dr Schlesinger choose to hit out so strongly and controversially in his first public pronouncements as head of the AEC? Perhaps one answer lies in his statement that "it is to industry's long run advantage that the public has high confidence that the AEC will appropriately perform its role".

SPACE

Martian Accord

by our Washington Correspondent

THE expected simultaneous arrival in orbit around Mars of NASA's Mariner-9 and the USSR's Mars 2 and 3 probes next month will set the stage for a far-reaching agreement on space matters between the United States and the Soviet Union. The fruit of a series of meetings in Moscow in August, the agreement provides for exchange of information between NASA and the Academy of Sciences of the USSR almost as soon as the information is relayed back to Earth from the Mars spacecraft.

This exchange of information will, however, be merely the first tangible evidence of a number of agreements hammered out between NASA and the Academy of Sciences of the USSR in recent months. The agreements, the terms of which have recently been made public by NASA, provide for cooperation on space activities ranging from planetary exploration to monitoring the Earth's environment by satellite.

The exchange of Mars data will allow particularly interesting phenomena to be studied by the spacecraft of both countries, and it will also allow some areas of Mars to be studied simultaneously by two probes. The idea is that experimental data will be relayed between NASA and the Academy of Sciences of the USSR by telex, by giving the nature, time and location in agreed Mars coordinates of phenomena such as changes in the colour of the Martian surface, or movements in the atmosphere. It is hoped that this exchange of information will be followed by a bilateral discussion of the preliminary results of the missions in March next year, and by a deeper review of the results a year later.

If this agreement is successfully carried out, it could pave the way for implementation of the other agreements reached in Moscow last August, and it could also help the negotiations aimed at development of compatible docking systems on US and USSR spacecraft (see *Nature*, **233**, 160; 1971). The fount from which this new accord springs is a meeting on space cooperation, held jointly by NASA and the Academy of Sciences of the USSR in January this year, as a result of which a number of joint working groups were set to work on areas thought to be particularly ripe for cooperative efforts. Recommendations from three of these working groups have recently been approved by NASA, and they provide for a variety of means for sharing information, and even for joint planning of some projects.

As far as exploration of near Earth space is concerned, one working group recommended that joint seminars be held whenever the need arises, to analyse data and coordinate programmes. On a slightly more practical note, the working group also suggested that a study be made of the possibility of transmitting magnetometer data from a USSR ground-based station, to the proposed US ATS-F geostationary satellite. The idea is that these data would then be relayed back to Earth with data from an on-board magnetometer and used for correlating magnetic variations in space with those on the ground.

The working group also recommends the holding of bilateral discussions on the results, objectives and strategy for planetary exploration and, in addition to sharing information on the Mariner and Mars 2 and 3 probes, that by the end of the year, the USSR will provide NASA with 700 time-of-flight data points for Venus, in exchange for about 900 post-1965 data points on Mars. On lunar exploration, representatives of the USSR will attend the Lunar Science Conference in Houston in January, and the working group has suggested that a lunar conference be held in the USSR in February or March 1973 so that future lunar exploration can be discussed. The agreement also provides for the exchange of photographs and maps of the lunar surface, and finally, the working group recommends that the two organizations continue to discuss the "possibilities of complementary lunar exploration activities".

To work towards a closer accord on environmental monitoring, NASA and the Academy of Sciences of the USSR have agreed to conduct remote sensing of designated sites in the US and the USSR either by satellite or by aircraft, and to exchange the results in the form of technical reports. The agreement also provides for exchange of infrared temperature measurements taken over the oceans, so that these measurements can be compared with actual measurements taken on board ships, and both organizations have agreed to work towards cooperation on programmes designed to monitor sea surface roughness, ocean biological productivity and sea ice conditions.

Finally, on meteorology, NASA and the USSR Academy of Sciences have agreed to exchange technical notes on temperature sounding by satellite, and to organize a joint experiment on temperature sounding over Western Europe for 1973-74. One NASA official said last week that the working group recommendations have all been accepted in principle by NASA and the Academy of Sciences of the USSR, but the practical details still need to be

sorted out. What happens in orbit around Mars could therefore be important to future cooperation on all kinds of space activities.

COMSAT

For Services Rendered

by our Washington Correspondent

THE Communications Satellite Corporation (Comsat) has been undercharged to the tune of \$6.1 million by the US government for the launching of communications satellites between 1965 and 1969, according to an investigation carried out by the General Accounting Office. The GAO recommends that \$31,000 should be recovered from Comsat, and that the basis on which the company should be charged for all the Intelsat IV launches (including the first launch last January) should be changed so that all the costs are included. NASA and the Air Force (which launches satellites for Comsat) are in general agreement, but the Department of State and Comsat itself do not see eye to eye with the GAO's recommendations.

Chief reasons for the undercharging were deficiencies in the accounting methods used by the Air Force and an agreement between NASA and the Department of Defense to the effect that Comsat would not be charged for research and development associated with the launches. The upshot was that Comsat entered into an agreement with NASA which left the US Government paying for both the depreciation and the research and development associated with the launching of Intelsat satellites. The GAO report suggests that the basis on which Comsat was charged for the launching of Intelsat I, II and III cannot now be changed, but the company was not charged for at least \$31,000 worth of services covered by the agreement. The GAO believes that this amount should be recovered, and that the agreement to launch Intelsat IV satellites should be renegotiated to include full launch costs.

The issue is complicated for several reasons. First, the Air Force, which launches the satellites, charges NASA, which in turn charge Comsat for the launch and support services. The basis for these bills is set out in agreements between NASA and Comsat, but the Intelsat IV agreement includes a clause to the effect that changes in the Air Force charges may be renegotiated. On the other hand, Comsat is the managing company for the International Telecommunications Satellite Consortium—a group of 80 nations interested in telecommunications satellites—which has had some severe internal problems during its first few years of existence.

The Department of State is therefore chary of causing an international dispute by an unseemly wrangle over \$31,000.

The GAO argues that special services provided by the US government should be charged at their full cost, and that Comsat is now a viable commercial concern which ought to pay its full share (it recently reported a net income for the first quarter of 1971 of \$4,978,000). A Comsat official said last week that the GAO report is being considered by the company, and pointed out that \$31,000 should be compared with the \$30 million it costs to launch each satellite. Comsat would, however, resist any attempt to change the basis for charges incurred by the launching of Intelsat IV satellites.

TUBERCULOSIS

Ribicoff lashes CDC

by our Washington Correspondent

SENATOR Abraham Ribicoff has, in the past few weeks, shown little respect for the agencies which he regulated when he was Secretary of Health, Education and Welfare in 1961-2. Two weeks ago, he lashed the NIH's Division of Biologics Standards for allegedly relying on dubious techniques for testing influenza vaccines, and last week the Food and Drug Administration and the Center for Disease Control were the subjects of his wrath for the part they played in the mass TB immunology programme on Capitol Hill last year, during which two people died of hepatitis. Senator Ribicoff's chief complaint is that the Center for Disease Control, which supervised the programme, gave 300 mg single doses of isoniazid—the drug commonly used for treatment and prevention of tuberculosis, instead of the usual treatment of three separate 100 mg tablets.

Based on an investigation conducted by the General Accounting Office, Senator Ribicoff's allegations were made in a stinging speech delivered in the Senate. He said that in 1964 the Center for Disease Control asked for permission to start investigational use of a 300 mg tablet of isoniazid. Under FDA rules, the centre should have supplied information derived from animal tests, and an outline of the planned investigation. "CDC supplied virtually none of this information to the FDA," Ribicoff claimed, "Instead, the CDC doctors started an accelerated experimental programme on a wide range of human subjects." Part of this experimental programme included the mass treatment of Capitol Hill employees last year after some cafeteria staff had contracted tuberculosis.

A spokesman for the FDA last week, however, although admitting that the agency "did not push the CDC for its

experimental results", suggested that the CDC's action in the Capitol Hill programme simply followed accepted medical practice. He also pointed out that the two people who died from hepatitis during the programme showed symptoms of the disease eight months after they began taking the drug. Nevertheless, the latest bulletin of the FDA points out that it has recently changed the labelling on isoniazid to the effect that the drug should not be used if

hepatic disease is suspected, and that the patient should be closely monitored. Alongside the announcement, however, the FDA bulletin contains an editorial by Gordon M. Meade, formerly medical director of the American Thoracic Society, who points out that "isoniazid remains a powerful and very effective antituberculosis agent which merits continued therapeutic and prophylactic use even though liver dysfunction can be associated with its use".

Short Notes

by our Washington Correspondent

Fort Worth

A CONFERENCE committee set up to decide the future of the Public Health Services Hospitals in general, and the drug rehabilitation centres at Lexington in Kentucky and Fort Worth in Texas in particular, reached deadlock on October 7. The Department of Health, Education and Welfare, frustrated in its plans to transfer the Fort Worth facility to the Bureau of Prisons, seized its chance and ordered the facility closed and all patients sent back for treatment in their home communities within twenty-four hours. The result: Congress was presented with a *fait accompli*, and, according to Mr James C. Wright, Texas representative in the House of Representatives, at least half of the ninety-two addicts discharged were taking drugs again before the weekend was finished.

The trouble was that in its desire to transfer the facility to the Bureau of Prisons, the Department of Health, Education and Welfare overlooked the fact that October 8 was a Friday. Consequently, many of the addicts could not be reached by representatives of their local rehabilitation centres for nearly three days, and not surprisingly many returned to the habit—none had been under treatment for more than four months. Mr Wright, who set his committee investigators to look into the fate of the addicts, found one pushing drugs in New Orleans; another smashed up a car and was jailed for speeding

and was later found to be high on drugs. Mr Wright described HEW's actions as "a flagrant breach of good faith with Congress". He said, "I was stunned that a Federal agency would stoop to using drug victims as pawns in a power play to thwart the will of Congress."

Science in School

FIGURES released this week by the National Science Foundation show that the number of schoolchildren in public schools opting for science subjects increased by a factor of 2.5 between 1948-49 and 1969-70—faster than total enrolment which for grades 9 to 12 increased 2.3 times over the same period. "The steady proportional growth of enrolments in such basic core courses as introductory algebra, introductory geometry, biology, and chemistry indicates a basically healthy state of science interest at the high school level," the NSF claims. The figures released, however, unfortunately include no discussion of recent trends which may reflect the growing disillusionment with science and technology in the present state of concern about the environment, and the NSF's optimism is therefore difficult to question. One fact which mars the optimism is that enrolments in physics have lagged dramatically during the past two decades—in 1949, 28 per cent of the 12th grade were enrolled in physics courses, but in 1970 the percentage had dropped to 18.

Table 1 Enrolment in Selected Science and Mathematical Courses in Grades 9 through 12 in US Public Secondary Schools as a Percentage of Total Enrolment in the Grade in which the Course is Usually Offered

Course	Grade in which usually offered	1948-49	Per cent 1960-61	1969-70
Natural sciences				
Biology	10	71	85	96
Chemistry	11	33	39	39
Physics	12	28	23	18
Selected social sciences:				
Economics	12	25	17	32
Sociology	12	18	17	19
Psychology	12	5	8	13
Mathematics:				
Introductory algebra	9	64	66	75
Introductory geometry	10	40	45	46
Adv. mathematics	11-12	27	32	31

Source: National Science Foundation adapted from Office of Education data.

NEWS AND VIEWS

Cholinergic Receptor Sites in Muscle

THE transfer of excitation from nerves to muscle fibres is the most thoroughly studied example of chemical transmission in the nervous system. A great deal is now known of the mechanisms responsible for the synthesis, storage, release and subsequent metabolism of the transmitter substance, acetylcholine, which is involved in the transmission of impulses at neuromuscular junctions. Until recently, much less was known of the post-junctional receptor mechanisms which are activated by acetylcholine to cause excitation of the muscle fibres. The exploitation of the extraordinary properties of the substance α -bungarotoxin, however, seems likely to lead to rapid progress in understanding the nature of such receptors.

Bungarotoxin is a protein, of molecular weight 8,000, which can be isolated from the venom of the Formosan snake *Bungarus multicinctus*. The protein seems to be the affinity label *par excellence* for acetylcholine receptors in vertebrate muscle, and in analogous structures such as the electric organs of electric fish and eels. The snake seems to possess a "receptor probe" molecule with near ideal properties, because bungarotoxin seems to bind with extremely high affinity and almost complete specificity to muscle and electric organ cholinergic receptor sites.

By using bungarotoxin labelled with radioactive iodine-131, it has already proved possible to perform preliminary studies towards the isolation and purification of cholinergic receptor molecules from electric organ tissue (Miledi, Molinoff and Potter, *Nature*, **229**, 554; 1971). In this issue of *Nature* (page 599), Miledi and Potter describe the application of bungarotoxin to studies of cholinergic receptors in amphibian and mammalian muscles. The extensive pharmacological studies of Chang and his colleagues in the past decade had shown that bungarotoxin is a powerful cholinergic antagonist in vertebrate muscles. Miledi and Potter confirmed that the exposure of frog or rat nerve-muscle preparations to low concentrations of bungarotoxin (1 $\mu\text{g}/\text{ml}$.) abolished neuromuscular transmission within a few minutes. The action of the toxin was irreversible, because no recovery of transmission or responses to applied acetylcholine occurred even after washing such preparations for up to eight days.

When nerve-muscle preparations were exposed to ^{131}I -labelled bungarotoxin, some label became firmly bound to the muscles; this binding was saturable and reached plateau levels within about two hours. If the binding of bungarotoxin is specific for cholinergic receptors, it should occur only on those areas of the muscle surface which underlie nerve terminals, because it is well known that non-innervated regions of muscle are virtually insensitive to acetylcholine. In diaphragm muscle, the innervated regions of muscle can be fairly simply dissected from non-innervated regions, because the neuromuscular junctions of adjacent muscle fibres are quite precisely aligned in such preparations. When such dissection was performed it was found that more than 90 per cent of the

bound labelled bungarotoxin was present in the innervated region of muscle, which represented only one sixth of the muscle weight. This high degree of localization of toxin binding to innervated regions of muscle speaks strongly in favour of the specificity of such binding to receptor sites.

Assuming that labelled bungarotoxin does bind with such specificity, then the amount of toxin bound under saturating conditions can be used to estimate the number of cholinergic receptor sites in various muscles. Using this approach Miledi and Potter obtain values of 10^9 sites per neuromuscular junction in frog sartorius muscles, and between 1 and 4×10^7 sites per junction in mouse and rat diaphragm muscles. The dimensions of the area of muscle membrane underlying nerve terminals are known from previous studies so that the density of receptor sites in frog sartorius junctions can be estimated as approximately 100,000 per square micron of membrane. The recent neurophysiological studies of Katz and Miledi (*Nature*, **226**, 962; 1970) suggest that the depolarization of the muscle membrane which occurs in response to nerve stimulation is composed of many virtually simultaneous "single shot" activations of cholinergic receptors. Katz and Miledi have estimated that each individual receptor activation or "single shot" event gives rise to a depolarization of 0.22 μV amplitude; to cause the overall depolarization observed in response to a single nerve impulse would require approximately 600,000 such events in the frog sartorius muscle. Even if as many as ten receptor molecules were involved in each "single shot" event, the calculated receptor population at frog sartorius junctions of 10^9 suggests that only a minute proportion of these receptors are activated by each nerve impulse.

The examination of bungarotoxin- ^{131}I binding in muscles which had been surgically denervated has also yielded important information on the changes in cholinergic receptors which occur in such preparations. Previous studies by Miledi (*J. Physiol.*, **151**, 1; 1960) and by Axelsson and Thesleff (*J. Physiol.*, **147**, 178; 1959) have shown that a curious spread of acetylcholine sensitivity occurs in muscles which have lost their normal innervation. In normal muscles only the region of membrane directly underlying the neuromuscular junctions responds sensitively to applied acetylcholine; denervation is followed by a gradual spread of this area of acetylcholine sensitivity radially over the muscle membrane, so that after several weeks the entire surface of the muscle membrane may become highly sensitive to externally applied transmitter.

Using labelled toxin, Miledi and Potter have now confirmed that a large increase in toxin binding sites occurs after surgical denervation of muscles; increases of up to twenty times the normal binding were observed three weeks after denervation. Furthermore, by dissection of innervated and non-innervated regions of diaphragm muscles it was found that toxin binding sites became uni-

formly distributed along the muscle fibres in the denervated preparations. Regions of muscle which are not normally innervated showed increases of up to 200-fold in their ability to bind bungarotoxin after denervation. When denervation was effected by crushing the motor nerves, damage to the muscle innervation is quite rapidly reversible, as the nerves will regenerate to re-innervate the muscle fibres within a few weeks. When such re-innervation occurs the changes in acetylcholine sensitivity of denervated muscles are reversed, and the preparations revert to their previous state of precisely localized acetylcholine sensitivity. Miledi and Potter have found that the spread of toxin binding sites is also reversed when denervated muscle preparations become re-innervated after nerve crush—showing again that the behaviour of bungarotoxin exactly parallels the inferences previously drawn about cholinergic receptor sites from neurophysiological evidence.

A curious and still mysterious aspect of the results reported by Miledi and Potter concerns the differences which seem to exist between the binding of bungarotoxin and curare to muscle receptors. Curare has for long been known as the classical antagonist drug for muscle acetylcholine receptors. Small concentrations of *d*-tubocurarine completely abolish neuromuscular transmission and cause total block in responses to applied acetylcholine. When curare was used in the presence of labelled bungarotoxin, however, it prevented the binding of only 50 per cent of the toxin, even when high concentrations of *d*-tubocurarine were used. The suggestion that some 50 per cent of toxin binding might be unspecific seems to be ruled out by the precise localization of toxin binding sites at neuromuscular junctions. It would seem therefore that curare can interact with only half of the receptor sites present in muscle; the nature of the difference between the curare-sensitive and insensitive sites remains obscure.

The results obtained by Miledi and Potter throw some light on the phenomenon of receptor desensitization. Previous studies have established that prolonged exposure of muscle pre-

paration to high concentrations of acetylcholine leads to a long lasting decrease in the ability of the muscles to respond to acetylcholine. Such desensitization could be attributable to a variety of causes. The receptor mechanism, for example, might retain the ability to bind acetylcholine, but failure might occur in the subsequent events leading to increased membrane permeability to ions. Miledi and Potter have now shown, however, that after prolonged exposure to acetylcholine the binding of labelled bungarotoxin is reduced by as much as 80 per cent. This suggests that the desensitization pheno-

menon involves some persistent change in the conformation of the receptor molecules.

Finally, Miledi and Potter report that the use of labelled bungarotoxin may be applied to muscle receptor isolation, as previously reported for the electric organ. Labelled toxin can be used to follow the isolation and purification of the toxin-binding substance. Preliminary experiments show that the labelled material can be solubilized from muscle preparations by the use of 'Triton X-100', and that the solubilized material can be further disaggregated by sodium dodecyl-lauryl sulphate.

EVOLUTION

Natural Selection and Industrial Pollution

from our Population Genetics Correspondent

ONE of the problems for many species of animals and plants is how to adapt to man's rapidly changing industrial environment. Until about 1800, man can have had only a slight effect on the environments of most species. Organisms evolved in response to the evolutionary changes of others and the generally slow changes of the physical environment. There was usually time for even the slowest breeders to adapt themselves to new conditions. But in the past few hundred years many species have become extinct and many more are threatened. This is a direct effect of man's activities.

An animal can adapt itself to a new environment provided the change is not so drastic that a large proportion of the population is eliminated by selection and not replaced by reproduction. Genetic variations in fitness are also necessary, of course, if fitness is to increase in the new environment. For an animal with a long generation time, the environmental change, if it is very rapid, may have gone so far in a single generation that the population cannot adapt to it. But an organism that produces many generations in the same period will easily be able to adapt to the relatively slight changes that take place in each of its brief generations. This is the problem with the use of pesticides. The pests are often organisms such as insects which produce a new generation every few weeks and they can usually adapt to the increasing quantities of insecticides the farmers throw at them. But the larger predators, such as birds of prey with their long generation times, are in danger of being completely eliminated. When predators are eliminated, their prey, if they are highly resistant to the pesticide, may increase rapidly and produce population explosions because there may be nothing but

disease and lack of food to check them. The biological environment thus becomes very unstable and greater and greater use of an increasing variety of pesticides is necessary to control the pests.

Pesticides are merely the most drastic of the selective agents produced by man. Many other selective agents are produced unconsciously as a byproduct of the industrial society. The evolution of industrial melanism in various moths is a well known example of selection favouring a dark variety of the species which will escape the attentions of predators in industrial environments. The selective forces in favour of the melanics are quite strong—of the order of a 50 per cent advantage to the melanic form. The value of smoke abatement is demonstrated by the almost immediate decline in the frequency of the melanics where smoke control zones are introduced (Clarke and Sheppard, *Proc. Roy. Soc., B*, **165**, 424; 1966).

Many other species of insect show examples of melanism, for example, the two-spot ladybird (*Adalia bipunctata*). The melanics, which are black with red spots, are found in high frequency in industrial areas. But the melanism cannot have evolved by selection by predators, for ladybirds are poisonous and are rejected as food. Presumably the melanics are simply more tolerant of the toxic elements in industrial pollution. Creed (in *Technological Injury*, edited by J. Rose; Gordon and Breach, 1969) certainly found a very close correlation between the frequency of melanic ladybirds and smoke concentration. In the latest issue of *Evolution* (**25**, 290; 1971) he describes the results of a survey of the frequencies of melanic ladybirds in various parts of Birmingham and compares them with their frequencies of a few years before.

In some areas of the city, the frequency dropped by as much as 50 per cent in four years. On average, from 1962 to 1967, the frequency of the black ladybirds dropped from about 45 per cent to 25 per cent. In the same period, as a result of the introduction of smoke control zones the level of smoke pollution fell by 40 per cent. There is no direct proof that selection for black ladybirds is caused by smoke pollution, but the circumstantial evidence is very strong.

Assuming that smoke abatement is responsible for the decline in the melanic ladybirds, it is possible to work out from Creed's figures the selection against the melanics in the less polluted environment of Birmingham. If about eight generations have passed since the introduction of smoke control zones, the selective coefficient against the melanics comes out at about 0.12. This means that the melanics suffer a mortality of 12 per cent over and above that of the normal, non-melanic ladybird in the new environment.

Although these selective forces are much smaller than those produced by pesticides, it is important to remember that they are the selective forces involved in the change back to a more natural environment. The original selection for melanism may have been much more drastic. Ladybirds can obviously adapt themselves to these industrial changes without much difficulty. Birds and mammals may not find it so easy. If the partial abatement of one aspect of industrial pollution can have such selective effects in thus raising the fitness of the normal red form of the ladybird, there can be no doubt of the benefit of the abatement of pollution in general in improving the natural environment. The leading article in the issue of *Nature* for October 15 (233, 437; 1971) made the point that pesticides have brought great benefits to man, as well as a certain amount of harm to the environments of many species: on balance "DDT may be good for people". Smoke and other avoidable effluents seem to offer no worthwhile benefits in compensation.

ANIMAL BREEDING

Keeping Fossils Alive

from a Correspondent

FOR ten years an *ad hoc* group of biologists has laboured to gain support for a scheme that would systematically conserve breeds of farm animals that are in danger of extinction. Several breeds of cattle, sheep and poultry were kept in a "gene bank" at Whipsnade (*Nature*, 202, 131; 1964) but these had to be dispersed when the Zoological Society of London needed the space.

In spite of lack of funds, some of the livestock were taken over by the Royal Agricultural Society of England at its National Agricultural Centre, Kenilworth. The organizing group became a working party under the auspices of this society, and it organized a conference on rare breed survival on October 15 to draw attention to the problem.

Mr W. Longrigg (Chief Livestock Adviser, MAFF) outlined the decline of breeds, which has chiefly been caused by changes in demand. Belatedly, geneticists are beginning to realize that the scientific approach to animal production should not be governed by current profitability, but by the need to maintain variability for future selection.

But economic value is only one aspect of the case for preservation which was presented by Dr P. A. Jewell (University College, London). He pointed out that every breed, like a wild species, represents unique genetic material of importance in archaeological, historical and scientific research as well as being a living museum of educational value. Once lost, a breed cannot be re-created. Dr Jewell described work in which bones from surviving primitive breeds such as the Soay sheep had been used in archaeological comparisons, and outlined research on the blood types of cattle in which primitive survivors had widened the range of study. He pointed out that Darwin used observations on domestic animals in his theory of evolution.

Dr M. L. Ryder (Animal Breeding Research Organization, Edinburgh) showed how survivals of sheep from prehistory, the Middle Ages and the nineteenth century had enabled a study of their development, and particularly of fleece evolution, to be carried out.

Professor J. C. Bowman (University of Reading) gave the results of a survey carried out in 1970 to ascertain the exact status of breeds. It was found that some well known sheep breeds had declined alarmingly to numbers lower than other breeds already designated "rare", for example, the Wensleydale (260) and Wiltshire (400) compared with the "rare" white-faced woodlands (700). He considered that it should be possible to keep a breed viable with as few as fifty individuals, provided the ratio of males to females was kept much higher than in normal husbandry. But two breeds of pigs (Tamworth and middle white) had fewer than the critical number of ten males.

The papers created considerable discussion and comment and it was decided to establish a society for the preservation of rare breeds, the functions of which would include the registering of animals, exchange of stock and technical advice. Further information can be gained from the Royal Agricultural Society of England.

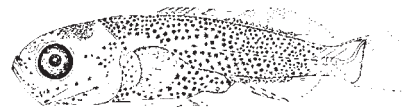
GOBIES

Two Species or One?

from our Marine Vertebrate Correspondent

THE diminutive goby *Lebetus* has attained some degree of distinction among European fish as the smallest vertebrate in the fauna, a claim which has even ensured it a place in the *Guinness Book of Records* (although parts of the entry are sadly erroneous). Now, however, its interest is reinforced by a remarkable uncertainty as to the number of species within European waters.

Neclâ Demir and F. S. Russell have reported recently (*J. Mar. Biol. Ass. UK*, 51, 669; 1971) that two forms of *Lebetus* postlarvae were distinguished in the collections of young fish at the Plymouth Laboratory. These two types of postlarvae show certain differences in the concentration of pigment cells, and also show quite distinct differences in fin development at comparable sizes. Thus in one form the dorsal and anal rays are well defined when the young fish is 5.0–5.5 mm long but are only just noticeable in the other form at this length. Pelvic fin development also shows similar differences, and even more significant are the numbers of vertebrae, 25–26 in one form, 27–29 in the other.



A drawing of one of the largest specimens of the Fage form of *Lebetus* postlarvae, 8.1 mm long.

The interest in this discovery, paradoxically, is that it confirms what was discovered by two other workers almost half a century before. In 1919 the Danish marine biologist C. Petersen identified the postlarvae of a goby as *Lebetus scorpioides*, and a year before L. Fage had found postlarvae of the other known species, *L. orca*. Both species had been described from Scandinavian waters in 1874 by Collett. Later work by A. V. Tåning on *Lebetus* from Iceland and Faroes confirmed that the postlarvae of the two named species could be distinguished in several ways, notably in their length at development of certain features.

It was thus widely accepted that there were two species of *Lebetus* in European waters, an assumption which was demolished by P. Miller in 1961 (*Nature*, 192, 675; 1961) who postulated that the two named species were sexually dimorphic forms of the same species. His hypothesis was exhaustively reinforced in a later paper (*Bull. Brit. Mus. (Nat. Hist.)*, 10, 205; 1963), in which he showed that having examined more than eighty specimens of full grown *Lebetus*.

including the types of both species, and dissected much of the material, he could find only male specimens of *L. orca*, and immature male and female *L. scorpioides*. This seemed to prove a classic case of sexual dimorphism in *Lebetus* (and other gobies are known to be sexually dimorphic), and Miller's conclusion has been generally accepted.

The work of Demir and Russell, however, has shown that *Lebetus* cannot be dismissed as a monotypic genus, for in addition to the morphological differences they have demonstrated biological differences. Collections made in 1970 of young fish in the western English Channel revealed forty-two "Petersen type postlarvae" and more than four hundred of the "Fage type", and it was established that the Fage type were more abundant between February and August, whereas the former were found from June to September only. The 1970 survey also showed that Petersen postlarvae were most common inshore; the other form was caught only at offshore stations.

It now seems therefore that there are two *Lebetus* species in the European Atlantic. If Miller's view of *L. orca* as a synonym of *L. scorpioides* (the only two named forms in the genus) is correct, then clearly one of the forms described from postlarvae by Demir and Russell is so far unrecognized and unnamed as an adult. On the other hand, it may be that both *L. orca* and *L. scorpioides* are good species, and if so it is to the credit of the earlier workers, Collett, Petersen and Fage, that their observations have been supported by this recent work on the postlarvae of *Lebetus*.

PLANT PATHOLOGY

Host-Parasite Contact

from a Correspondent

THE need for studies, on very short time scales, of the reactions arising from host-parasite contact was repeatedly stressed during a symposium on fungal pathogenicity and the plant's response, held at Long Ashton Research Station, University of Bristol, from September 22 to 24.

In his inaugural lecture, Professor R. K. S. Wood (Imperial College, London) emphasized the importance and specificity of the hypersensitive response (using the term in its botanical context). Originally applied to the death of cells in an incompatible relation between an obligate pathogen and a resistant host, it is now clear that the general concept may be more widely applicable. Of particular interest in this context was the demonstration by Drs A. Kaars Sijpesteijn and A. van Dijkman (TNO Biochemical Department, Utrecht) of the effect of two near-isogenic races of *Cladosporium fulvum* in causing leak-

age from cells of two tomato cultivars, which showed complementary host-parasite reactions. Other contributions were devoted to the nature of the trigger system which initiates the biochemical and structural reactions that occur when a plant is challenged by a potential pathogen. The complexity of compounds implicated in disease resistance makes it difficult to resolve the sequence of cause and effect.

The intensive studies during the past 30 years on the role of ethylene were reviewed by Dr E. C. Hislop (Long Ashton Research Station) who drew attention to the possible implications of ethylene in altering the rate of disease development, and Dr Barbara Lund (Food Research Institute, Norwich) demonstrated that bacterial enzymes probably activate the production of ethylene by a route already present in normal tissue.

By original definition, phytoalexins are absent in the unchallenged host plant but the symposium suggested no such clear-cut distinction between preformed and induced resistance factors.

Because obligate pathogens cannot be grown in axenic culture, it is particularly difficult to examine the pathogen component of the host-pathogen system. The use of tissue culture has been of some value but, as shown by Dr D. S.

Ingram and Dr Inez Tommerup (University of Cambridge) with *Peronospora farinosa* on sugar beet callus tissue, it is not necessarily typical of the system *in vivo*. Electron microscopy has been increasingly used to study the structure of the host pathogen interfaces, where interchanges of nutrients, regulatory compounds, proteins, and perhaps even genetic material must occur. The participants were impressed by the elegance of such ultrastructural studies seen in the illustrations of Professor P. H. Williams (University of Wisconsin), Professor C. E. Bracker (Purdue University) and Dr L. J. Littlefield (North Dakota State University), Professor Williams showing how, in cabbage clubroot, the bullet-like "stachel" from encysted zoospores penetrate in a matter of seconds, but minutes are apparently required for the host to respond.

In his closing remarks, Professor P. W. Brian (University of Cambridge) considered that the present era of plant pathology has the same advancing frontiers as molecular biology and plant biochemistry. The study of host-pathogen interactions has not so far aided the chemical control of plant disease, but the symposium illustrated the delicacy of the balance of such interactions, better knowledge of which may lead to new ways of combating the pathogen.

Mutating the Rous Sarcoma Provirus

FURTHER evidence, if any were needed, that the replication of RNA tumour viruses involves a DNA replicative intermediate, the provirus, is reported by Bader and Brown in next Wednesday's *Nature New Biology*. These authors have exploited the reverse transcription of Rous sarcoma virus genomes to obtain mutants of this virus which are temperature sensitive for transformation. They argued that if chick cells are exposed to 5-bromodeoxyuridine, the mutagenic analogue of deoxythymidine, when they are infected with Rous sarcoma virus some BUdR should be incorporated into the DNA provirus as it is synthesized, presumably by the reverse transcriptase in the infecting virus particles. Furthermore, when the DNA proviruses containing BUdR residues in place of some of their thymidine residues are in turn transcribed to yield progeny RNA genomes, guanine for adenine base substitutions should be induced and some mutant progeny Rous genomes would be expected.

Bader and Brown found that if chick cells are simultaneously infected with Rous sarcoma virus and exposed to BUdR, but not if the exposure to BUdR is 12 hours before or 12 hours after infection, temperature sensitive progeny

virus can indeed be isolated from the transformed cells some 24 hours after infection. Such progeny virus stocks transform chick cells more efficiently at 36° C than 40.5° C, whereas the converse is true of wild type Rous sarcoma virus. This observation immediately suggested that many of the progeny virus particles liberated from the cells exposed to BUdR contain temperature sensitive lesions in the gene or genes responsible for transformation.

To prove this point, Bader and Brown isolated the progeny virus produced by 119 clones of cells transformed in the presence of BUdR and found that of these 20 yielded mutant virus temperature sensitive for transformation. Because BUdR is not metabolized into RNA by chick cells this result must mean at the very least that that part of the Rous sarcoma genome which determines the ability to cause the transformation of chick fibroblasts must be replicated by way of a DNA intermediate. Moreover, because cells transformed by these mutants at their permissive temperature and then shifted to the nonpermissive temperature continue to produce progeny virus, the temperature sensitive lesion cannot be interfering with the replication and packaging of the mutant genomes.

LIPIDS

Protomembranes

from our Molecular Biology Correspondent

It seems that the study of hydrocarbons will for some time continue to provide a comfortable livelihood for many a physical chemist in the guise of a molecular biologist. The possibilities in liposomes are still far from exhausted, and McConnell especially has shown how much structural and dynamic information can be extracted from his spin-label experiments on such systems.

Cadenhead and Katti (*Biochim. Biophys. Acta*, **241**, 709; 1971) have tried to step on the cake by showing that a molecule carrying a large and ugly spin-label group will not necessarily have the same physical properties as the virgin form. There is no complete counter to this criticism, though it can be argued that inasmuch as spin-labelled lipids can be biosynthetically incorporated in membranes, they seem to be at least compatible, if not identical in behaviour, with normal lipids. Moreover the results of other methods, including NMR and X-ray diffraction, seem to be converging on much the same conclusions. Cadenhead and Katti, however, have looked into the effects of oxyloxazolidine-substituted cholesterol-like steroids on the surface properties of films, in which they are mixed with myristic acid. The polar spin-label group, they find, changes the effect on the surface isotherms considerably, compared with the parent compound; they suggest that this will generally be the case and recommend caution in visiting the results of spin-labelled onto unlabelled systems.

At the same time Birdsall *et al.* (*ibid.*, 693) offer support for the conclusions of Hubbell and McConnell concerning configurational freedom of lipid chains in bilayers. Instead of introducing the relatively large and polar spin-label, they have prepared a series of stearic acids containing a fluorine atom at various positions in the paraffin chain. These derivatives were then mixed with lecithin and dispersed in water as vesicles. NMR signals from the ^{19}F nucleus revealed that the line-widths changed drastically with the distance of the fluorine from the head group, indicating a large increase in freedom of motion going from the outside to the centre of the bilayer. This is by now a heavily laboured conclusion. It may be hoped, however, that a more complete analysis, including an estimate of exchange line-broadening effects, will make possible a quantitative treatment, so as to allow quantitative comparison with the results of other methods.

The most interesting current development in the application of NMR to the

study of such systems comes from the Moscow group (Bystrov *et al.*, *Chem. Phys. Lipids*, **6**, 345; 1971), who have examined the effect of paramagnetic perturbants on the proton magnetic resonance spectra of lecithin vesicles. When vesicles are prepared in water (or actually D_2O) containing manganous ions, there is a spectacular broadening effect, primarily of the signal from the quaternary amine methyl hydrogens, because these are in contact with the aqueous medium on the inside and outside of the vesicle. The ions do not penetrate the bilayer, however, so that when the vesicles are made in water and the paramagnetic ions are only afterwards added, a quite different effect is observed: superimposed on the shallow broadened background is a sharp peak of diminished magnitude. The polar heads on the inner surface are not in contact with the ions, and thus give rise to the sharp unperturbed resonance, whereas those on the outer surface generate only the broadened pedestal. When such preparations are sonicated, the residual sharp line from the internal head groups vanishes into the broadened background. An equally spectacular effect is observed with europium ions, which elicit a large contact-shift in the head-group reson-

ance, so that when they are added to preformed vesicles, two sharp signals, one from the internal, the other from the external head groups, are observed.

Bystrov *et al.* also prepared micelles of lecithin in benzene, and found that these could be made to take up water. Again the introduction of the paramagnetic perturbant perturbed only the head groups exposed to water on the inner surface. These experiments offer good promise for the separate observation of events at different surfaces both of models and of real cell membranes.

A review by Kuhn and Möbius (*Angew. Chem.*, **10**, 620; 1971) of their remarkable work on monolayer systems, and the use of excitation transfer to measure critical distances, will give workers in the biological membrane field much food for rumination. The scope of this work takes in many areas of chemistry, but some examples of biological interest have been included in the survey, in particular the measurement of membrane thickness by introducing the membrane between emitter and sensitive layers and measuring the efficiency of excitation transfer and the construction to order of various arrangements of lipid and protein monolayers.

Rescuing Mouse Sarcoma Virus

In next Wednesday's *Nature New Biology*, Klement, Nicolson and Huebner report that they have rescued mouse sarcoma virus particles from rat cells which although transformed by this virus do not support its replication until they are exposed to appropriate concentrations of 5-bromodeoxyuridine (BUdR).

This discovery, which follows hard on the heels of those of Rowe's group (*Science*, **174**, 155; 1971) and Aaronson, Todaro and Scolnick (*ibid.*, 157), who have used BUdR and IUdR to rescue respectively mouse leukaemia virus from non-producing AKR strain mouse embryo cells and a C-type virus, of which the oncogenic potential has yet to be tested, from normal BALB/C 3T3 fibroblasts, is a powerful shot in the arm for the devotees of the oncogene hypothesis of malignant transformation. And of course the induction with BUdR of IUdR technique provides a new approach to the search for other mammalian, including human, RNA tumour viruses.

The experiments of the three groups, which are essentially identical, indicate that the drugs BUdR and IUdR, for reasons that are far from clear at present, stimulate the active replication of dormant RNA tumour virus genomes in rat and mouse cells. The virus which

Rowe's group have activated in embryonic cells of the AKR strain, a high leukaemia strain, seems to be identical to that isolatable from adult AKR mice with the disease. The virus which Todaro's group have activated in 3T3 cells has all the vital statistics of a C-type RNA tumour virus and it will be fascinating to learn whether or not it is indeed oncogenic. And Huebner's group believe that the rescue of mouse sarcoma virus from transformed but non-producing rat cells is most probably a consequence of the activation of a dormant rat "leukaemia" virus which can provide the helper function required for the replication of mouse sarcoma virus and normally provided by mouse leukaemia virus.

The avian oncogenic virologists, and Weiss in particular, have, of course, already shown that a C-type virus, probably an avian leukosis virus, can be activated in the cells of domestic chicks and wild jungle fowl by various chemicals including carcinogens, and so the betting must be that all vertebrate cells have the genetic potential to specify C-type viruses at least some of which are oncogenic. And that is what Huebner and Todaro's oncogene hypothesis predicts; perhaps all cells do indeed carry genes which can render them malignant.

ESTUARIES

Inaugural Meeting

from a Correspondent

THE inaugural meeting of the Estuarine and Brackish Water Biological Association was held at the Zoological Society of London on October 13. After the formal founding of the association by Dr D. S. Ranwell (NERC Coastal Ecology Research Station), it was announced that the steering committee would act as the first council and that the constitution would be approved at the first annual general meeting in April 1972. Some participants suggested that the word "biological" should be deleted from the name of the association, because the association plans to encourage an interdisciplinary approach to studies of estuaries. Mr R. Bassindale (University of Bristol) serves as the first president and Dr R. S. K. Barnes (Central Electricity Research Laboratories, Fawley, Southampton) is the secretary, from whom full details of membership may be obtained.

A symposium on the estuarine environment was held during the remainder of the meeting. The symposium was opened by Professor W. D. P. Stewart (University of Dundee), who reviewed the sites of estuaries in the United Kingdom and estuarine research projects which are in progress. Professor Stewart stressed the importance of estuaries in relation to man's activities and the need to develop management policies and a strategy for research.

Professor L. C. Beadle (University of Newcastle upon Tyne) discussed physiological adjustments shown by organisms in relation to extreme fluctuations of ionic conditions which may occur in estuaries. He stressed the need for information on the time which organisms take to adjust to salinity stress, how this affects growth rates and the extent to which changes in ionic fluxes may be used as indicators of salinity stress before more adverse effects of stress on organisms are apparent.

Mr A. J. Phillips (Central Electricity Research Laboratories, Fawley) stressed that although there is a considerable body of scattered information on the chemistry of estuaries, there is a lack of guiding principles. He also emphasized the differences in the chemistry of sea water and river water, not only in relation to ionic activities but also in relation to different ionic species present in the two types of solution. He suggested that the amounts of trace elements present in sea water and estuarine water may be underestimated by the use of some methods, because a fraction of these elements is bound to particulate matter carried in suspension. The particulate matter tends to flocculate in the presence of high salinities.

The last speaker in the morning, Dr

K. R. Dyer (University of Southampton), spoke on sedimentation in estuaries. Salinity levels are extremely important, besides topography, current flow and volume of water, in determining rates of sedimentation. Various degrees of mixing may occur between river and sea waters, resulting in the formation of completely stratified to completely mixed bodies of water. The principal minerals in sediments are illite, montmorillonite and kaolinite which have different fall velocities that in part are dependent on temperature and salinity. A mean sedimentation rate for estuaries in Britain is approximately 0.2 cm per annum. Under some conditions, fluid muds are present close to the bottom of estuaries which during ebb and flow tides may give rise to peaks in turbidity.

In the afternoon, Dr R. L. Jefferies (University of East Anglia) reviewed the research on salt marshes. He emphasized the high net productivity of marshes and suggested that plants growing under high salinities are often perennial, show vegetative reproduction and have a limited but rapid growth period. He also discussed the possibility of assaying adaptive enzymes such as nitrate reductase in plants in an attempt to measure the level of nitrate-nitrogen available for plant growth in enriched estuaries.

Dr P. R. Walne (Ministry of Agricul-

ture Shellfish Laboratories) spoke on the importance of estuaries to commercial fisheries. He concentrated on the shellfish industry which has an annual turnover of £0.5 million in Britain and examined methods for improving yield such as the moving of shellfish or encouraging sprat to settle.

Dr H. Milne (University of Aberdeen) examined the trophic relations at the upper end of the food chain in an unpolluted estuary, the Ythan in Aberdeenshire. Although the results are preliminary, he has been able to determine the annual energy budget of mussel populations in commercial beds and to show that predation by birds together with loss of energy as a result of metabolism during the winter cause the principal reductions of energy in the standing crop.

Mr M. J. Barrett (Water Pollution Research Laboratory, Stevenage) discussed changes in the oxygen levels in the waters of the Thames during a period of 80 years and how in the past 15 years conditions have improved so that anaerobic reaches are no longer present.

In the closing contribution, Professor D. R. Arthur (King's College, London) emphasized the need for interdisciplinary studies on estuaries and stressed the approach used by systems analysts in gaining a better understanding of estuarine systems.

Dilatons and Gravity

IN the continuing attempt to establish connexions between the microscopic and macroscopic worlds of physics, it is becoming fashionable to explore gravitation from the standpoint of elementary particle physics; and in next Monday's *Nature Physical Science*, Y. Fujii of the University of Tokyo describes a contribution to this approach. The starting point of his work is the prediction of the Goldstone theorem that the invariance of the world under scale transformations (dilatations) can be realized physically by the existence of a massless boson, known here as the "dilaton". Any breaking of scale invariance results in a non-zero mass κ for the dilaton, with a consequent finite range κ^{-1} for any physical effects. If the dilaton is now coupled to gravity, it would manifest itself by a modification of the usual Newtonian force law in the range κ^{-1} . The choice of κ is entirely arbitrary, but a characteristic mass may be constructed from gravitational and elementary particle parameters, and this leads to values of κ^{-1} of a few km or less.

Fujii's concern is to devise experiments to detect any such short range non-Newtonian component, if it exists. Several suggestions are made for laboratory and larger scale searches for this component. The chief difficulty with

all these experiments is to achieve sufficient sensitivity. The most direct method—the Cavendish experiment in its refined form—could almost detect a component with $\kappa^{-1} \sim 10$ m. An amusing terrestrial experiment is also suggested in which the change in gravity due to the lowering of the water level in a large semicircular reservoir is measured. Other terrestrial approaches would be to compare the size of the Earth as measured by astrogeodetic techniques with the radius calculated from the gravitational force at the Earth's surface. Or the variation of gravity with altitude could be explored. Although astrophysical considerations might be expected to be the most appropriate for testing gravitational theories, only one extra-terrestrial experiment is mentioned, that of observing the behaviour of artificial lunar satellites which orbit close to the surface of the Moon and would be perturbed by a short range force.

Unfortunately, in all these large scale experiments it is impossible to make correct allowances for the many other complicated effects, such as those due to inhomogeneities beneath the terrestrial and lunar surfaces. Nevertheless, Fujii places present limits on κ^{-1} of either between 10 m and 1 km, or less than 1 cm.

PEAT

Pennsylvanian Deposits

from a Correspondent

WETLAND ecosystems are particularly prone to human interference. Either they are drained for agriculture or they are exploited for peat, but rarely are they left untouched. The reason for this is probably that it is difficult to justify mire conservation except in terms of the intrinsic scientific interest of the habitat, which appeals only to a relatively small minority of the population. The interest of these habitats lies both in their distinctive wildlife and also in that they build up a record of their own development in the peat which they form. Peat is an accumulation of the remains of dead vegetation which once grew upon the surface of the mire. Waterlogged, anaerobic conditions discourage microbial activity so that decay processes become impaired. Such remains are often recognizable and document the vegetational succession on the site. Furthermore, the peat contains pollen which may have travelled considerable distances in the air before being deposited on the mire surface and from this can be reconstructed the vegetational history of much larger areas surrounding the mire. Such information can be valuable in the study of climatic changes in a region.

Peat has long been valued as an energy reserve, a fossil fuel of recent origin. But in recent times its value as a horticultural commodity has risen very considerably, a value which derives from certain of its physical properties. In the first place it is a source of organic matter which, when added to a well aerated soil, acts as an energy source for soil microbes and thus improves soil structure. The water holding capacity of peat is very high, its value depending upon the vegetation from which it was formed, for example, *Sphagnum* moss peat can hold 800 per cent of its dry weight in water, and for sedge peat the figure is about 400 per cent. Peat also has properties of nutrient retention which make it valuable when added with fertilizers to soil. This horticultural demand for peat has placed a new stress on the world's harassed wetlands.

A recent bulletin of the United States Geological Survey (C. C. Cameron, Bulletin 1317-A; 1970) is devoted to the peat deposits of the Appalachian Mountain region of north-east Pennsylvania. The author calculates that the resources of this 900 square mile area are more than 13 million short tons of air dried peat. This estimate is based on a study of ninety-five peat deposits most of which are less than 100 acres in area and less than 16 feet deep. In spite of the small size of these resources (between 10,000 and 100,000 tons each), the

proximity of the Atlantic seaboard and metropolitan markets for peat products makes their exploitation an economic possibility. In 1966 the United States imported peat to the value of \$11½ million. With a demand of this size it is understandable that the Department of the Interior should seek a home supply of peat. If economic needs demand the exploitation of an area such as north-east Pennsylvania, this should be accompanied by the conservation of selected sites representative of the wide range of mire types found in that area. In this way the irretrievable loss of valuable ecological information can be avoided.

PLANT GROWTH

Izmir Institute

from a Correspondent

"NEVER has so much been said by so many on a subject about which we know so little." This remark, which was made at the 1955 Wye College conference on plant growth regulation, is perhaps as true today as it was then. The NATO Advanced Summer Institute on the

same subject, which was held at Izmir in Turkey from September 20 to October 1, highlighted the complexity of the interactions between the classical auxins and the gibberellins, cytokinins, abscisic acid and ethylene. It also stressed the need for "translating" results obtained on segments of plants to whole plants.

The organizers arranged for a general review to precede the more detailed accounts of research on specific topics. This met one of the objectives of the NATO institute; that is, to provide students with a general survey of the field while at the same time providing a forum for research workers to present original work. Dr I. D. J. Phillips (University of Exeter) opened the proceedings with a review of the principles of promotion and inhibition of growth in plants. This was followed by an original paper by Professor E. C. Cocking (University of Nottingham) on specific discussion of growth in isolated protoplasts. Other contributions from the United Kingdom included reviews on the gibberellins (Dr J. Macmillan, University of Bristol), some recent studies on gibberellin-like substances in higher plants (Dr J. L. Stoddart, Welsh Plant Breeding Station) and growth regula-

How the Molecules of Collagen are Assembled

NEXT Wednesday's *Nature New Biology* will see the publication of the third article in the past few months on a long-standing problem in molecular biology; how the molecules of collagen are assembled in three dimensions to produce functional connective tissue. Each approach starts with different data and it is interesting that they all point to the same solution—a five-fold microfibril.

The latest study comes straight to the point that any answer must finally explain. How is the molecular packing related to the molecular structure? Segrest and Cunningham start with the tropocollagen molecule, a three-strand rope formed by supercoiling two identical helices termed α_1 and α_2 . They speculate that the primordial collagen may have contained three identical chains of general structure (gly-pro-X)-where X is apolar. Fibril formation would likely take place by more or less random parallel aggregation of such molecules. They then suppose that a mutation in the nucleic acid led to a charged side-chain at the same level in all three α chains, followed by a second mutation which resulted in the appearance of a second charge side-chain, complementary to the first and separated by a suitable distance along the molecule. If the relative positions of the two new complementary groups are correct, fibril growth may proceed by lateral accretion of staggered molecules.

In addition to appropriate position along the length of the molecule, several factors are involved in the correct relationship of mutations for fibril growth. When parallel cylinders are packed they touch along edges of contact. For helices, points which lie along such an edge must be separated axially by integrals of the pitch of the helices. Thus mutations can only occur along these edges for formation of a regular fibril. Segrest and Cunningham list the types of packing which could result from various mutation positions. One of these is a hexagonal array; another is an extended pleated sheet. The third is the five-fold microfibril suggested independently on other grounds. The two identical α_1 chains in the tropocollagen molecule are related by an aximuthal rotation of 108°, so that the existence of contact edge on one chain automatically generates an identical edge 108° round from the first. If complementarity of these edges can be obtained by an axial stagger, then a second molecule would add to the first at an angle of 108° which is the internal angle of a pentagon.

Model building has its limitations. Without further information it is frequently only able to indicate possible structures. It is gratifying to see how the microfibril suggested by X-ray diffraction and electron microscopy arises readily from the structure of the component molecules.

tion in a whole plant (Professor A. J. Vlitos, Tate and Lyle, Ltd, Keston), and some aspects of growth control in cereal seedlings (Dr L. L. Mer, Imperial College, London).

The transport of auxins, gibberellins and cytokinins was discussed by several participants. Professor W. P. Jacobs (Princeton University) presented evidence for the polar transport (in *Coleus*) of all of the three classes of growth regulators. Professor T. K. Scott (University of North Carolina) demonstrated that the movement of gibberellins in sugarcane tissues is strongly influenced by auxins and perhaps also by other endogenous growth regulators. Dr H. Kaldewey (University of Saarlandes) presented good evidence for polar movement of radioactive ^{14}IAA in the stems and in the roots of intact cotton seedlings. An original concept for explaining apical dominance was presented by Professor Y. Vardar (Ege University, Turkey). Professor H. Bärstrom (University of Lund) demonstrated quite conclusively that the anatomy (that is, cell type) of a given tissue determines to a considerable extent the velocity of movement of growth regulators through the tissue. He also demonstrated the histological effects on cells after treatment with various classes of growth regulators.

Interactions between the various classes of endogenous growth regulators were reviewed by Professor A. W. Galston (Yale University) and by Professor A. C. Leopold (Purdue University). Both speakers stressed that auxins, gibberellins, cytokinins, abscisic acid and ethylene operate in a dynamic system where the presence of one type of growth regulator influences the physiological activity of another. Auxin, for example, may predispose a cell to respond to a gibberellin, and in some instances treating a tissue with a gibberellin induces it to produce more auxin; in turn this may lead to a release of ethylene. Ethylene may produce a host of physiological responses including the induction of flowering in tissue cultures of higher plants as was demonstrated by Dr C. Nitsch (CNRS, Gif-sur-Yvette).

Dr O. M. Heide (Makerere University) reviewed some of the physiological effects of cytokinins with special reference to the induction of rooting and initiation of buds. He demonstrated that these effects were mediated by temperature. Of particular interest was the demonstration that rooting of cuttings can be stimulated by cytokinins under certain conditions.

The role of ethylene in plant growth was discussed in some detail by Dr S. P. Burg (University of Miami). As one delegate remarked, "Ethylene can do anything an auxin can do and even better". Dr Burg demonstrated that ethylene is

indeed a very active plant growth regulator but an extremely difficult and elusive compound to study, chiefly because it is a gas.

A thorough review of the auxins and other auxin-like substances by Professor K. V. Thimann (University of California, Santa Cruz), followed by a more specific contribution by Dr J. Shen-Miller (Argonne National Laboratory), set the stage for lively discussions on whether the classical auxins, as studied in isolated plant parts and in dark rooms, are as relevant to the growth of intact plants as was once thought. Dr Mer indicated, in discussion, that some of the published results may be interpreted in more than one way and that extrapolations to whole plant physiology from such results are invalid.

Inhibitors of growth, some new synthetic growth regulators and some interesting and aberrant physiological

effects of auxins on growth were also discussed. Dr K. Dörffling (State Institute for General Botany and Botanic Garden, Hamburg) reviewed the status of abscisic acid as a naturally-occurring plant growth regulator.

A thorough description of the synthetic growth regulators, known as morphactins, was presented by Dr G. Schneider (E. Merck AG, Darmstadt). These substances retard the growth and development of dicotyledonous species and in the future may have important practical applications.

Professor L. Lona (University of Parma) presented evidence that indole-3-butyric acid (IBA) acts as a gibberellin-like substance in certain species. He stressed the need for evaluating the physiological effects of any given growth regulator on a wide range of species before attempting to classify the regulator.

Heterogeneous Nucleation from a Polymer Melt

THE ingenious researches of David Turnbull in the nineteen-fifties stimulated a great deal of work on the mechanisms and temperature dependence of nucleation in supercooled metallic and molecular melts. The classical Becker-Volmer theory of homogeneous nucleation is now firmly established for these categories of materials, but at the same time it has become clear that nuclei form homogeneously only under very exceptional circumstances: normal melts contain specks of impurity that act as nucleation catalysts. *A fortiori*, crystals forming from polymer melts would be expected to be heterogeneously nucleated always, in view of the extreme improbability that a sufficient length of polymer chain should align spontaneously in the correct configuration to constrain further growth in the proper pattern. But so far no heterogeneous nucleation catalyst for a polymer has been properly characterized and, generally speaking, this aspect of polymer science is confused.

In next Monday's *Nature Physical Science*, S. Y. Hobbs casts some light on this problem. Hobbs crystallized spherulites of isotactic polypropylene from the melt, using short lengths of carbon fibre as nucleation catalysts. These were standard 'Morganite' fibres made from a polyacrylonitrile precursor, and two fibre variants were used—high strength (type 1) and high modulus (type 2). Type 1 fibre is known, from electron microscopy and small-angle X-ray scattering, to consist of very small turbostratic graphite crystallites of random orientation, whereas type 2 contains larger, highly oriented crystallites, with the basal

graphite planes parallel to the fibre axis. Hobbs found that type 2 fibres were highly efficient nucleation catalysts, whereas type 1 fibres were ineffective.

The polymer chains in the crystalline structure of polypropylene are arranged in straight spirals. Hobbs showed that such a spiral can be accurately keyed to a graphite basal plane through some of the hydrogen atoms which (according to recent molecular orbital calculations) are preferentially chemisorbed at the midpoints of C-C bonds in the graphite structure. For effective nucleation the spiral has to be "set", perhaps along one complete repeat period, and this requires the graphite basal plane which serves as substrate to exceed a certain minimum dimension, which Hobbs estimates at 50 Å. In type 2 carbon fibres, the constituent crystallites are more than 100 Å across measured in the basal plane, whereas in type 1 they are only ~25 Å across. The inefficacy of type 2 fibres is thus readily understood.

Type 2 carbon fibres have a crystallographic "fibre texture", which implies that the normals to the basal plane are randomly distributed in a plane normal to the fibre axis. It would be interesting to continue Hobbs's work with single crystals of graphite or alternatively with highly oriented pyrolytic graphite. On the basis of Hobbs's interpretation of his observations, polypropylene would be expected to crystallize on such a substrate in two-dimensional spherulites, all polymer spirals being constrained to lie parallel to the exposed graphite basal plane. This form of nucleation would generate a unique form of preferred orientation with, presumably, strongly anisotropic properties.

Outlook for Controlled Fusion Power

J. L. TUCK

University of California, Los Alamos Scientific Laboratory, Los Alamos, New Mexico

Fission and fusion are compared as a source of energy for the future. Energy reserves for the two are similar, as are their pollution and ecological characteristics. A remote integrated site policy is recommended for fission.

UNTIL now mankind's chief energy source has been fossil fuel. Within the next century, if the population continues its present growth, rising energy demands and falling fuel reserves will force us to find new sources of energy and, although several possibilities exist¹⁻⁴, it looks at present as if the chief energy source of the future is likely to be either nuclear fission or nuclear fusion.

Comparison of Fission and Fusion

The advantages of nuclear fission as an energy source are, first, that the fuel reserves are large. The fuel used in fission reactors at present is obtained from rich ores, at a cost of ~\$5.00 per pound of UO_2 , for which the reserve is small—a hundred years at most. But for weak ores (containing ~10 p.p.m. of UO_2), which would be uneconomic to extract at the present time, the reserve is large. Thus, using the customary energy unit $Q (= 10^{18} \text{ BTU} = 10^{21} \text{ J})$, the reserve at the 10 p.p.m. level will be about $10^6 Q$ if the uranium is used efficiently in breeder fission reactors. The present annual energy consumption rate of the world is $0.2Q$, and it has been estimated³ that for a world with an asymptotic population of 7×10^9 this might rise to 2 to $3Q$ a year. This asymptotic world has then about one million years supply of energy in its reserve of accessible fuel for fission.

The second advantage of fission is that non-breeder fission reactors are available and already producing power competitively. Breeder fission reactors exist only as prototypes at present but are in a state of intense development. They are expected to come into wide commercial use in the next decade and to be competitive power sources. The third advantage is that fission reactors pollute less than coal burning power plants.

The disadvantages of fission as an energy source are, first, that the plants are hazardous (because of possible melt down, power excursions and so on). But a major bomb-type explosion is probably impossible and precautions, involving heavy containment vessels and fail-safe cooling equipment, are taken to prevent escape of radioactive material into the atmosphere as a result of imaginable accidents. But no matter how safe they are made, they can probably never be made entirely safe from technically informed sabotage. It seems unlikely, therefore, that they will ever be installed within cities.

The second disadvantage is that the plutonium by-product is a material fundamental to nuclear weapons. In an asymptotic world running on fission, there would be enough to make tens of thousands of nuclear bombs a year. It follows then, that the rise to power of, say, a Hitler would assure a Götterdämmerung finale. Third, the plutonium is valuable, saleable, and therefore likely to be stolen. Mankind's past behaviour

suggests that, sooner or later, somebody will "cobble" a crude fission bomb—of a kiloton or so—and fire it in a city centre. Fourth, fission products are biologically hazardous and probably should not be transported over long distances for fear of dispersion by accident.

The first fusion plants will have to operate on the, thermodynamically speaking, less difficult, deuterium-tritium (DT) cycle. The factor determining fuel reserves will not be deuterium but lithium and the reserves of this are about $2 \times 10^6 Q$ or enough to last about 10^6 yr; ultimately fusion plants operating on the DD cycle and fuelled by deuterium may be achieved. Deuterium is present to the extent of 1 part in 50,000 by weight of the oceans of the Earth and constitutes an energy reserve of $10^9 Q$ —a fuel reserve for about 10^9 yr at the asymptotic Q rate. Second, fusion reactors are incapable of running out of control and it follows that they could, if necessary, be located inside urban complexes. They also generate no fission products and, although the tritium is valuable and subject to theft, it is not adaptable to the construction of illicit bombs.

The disadvantages of fusion are, first, that fusion power is not yet available; second, that it seems as if fusion reactors will be more complicated than the basically very simple fission reactor, though this may not necessarily always be the case and it is too early to be sure. This suggests, however, that it will take time for fusion power to become competitive with fission. Third, although a fusion power reactor produces no long term radioactive fission products, it will produce about ten times as many neutrons kWh^{-1} as a fission reactor. This means that a high level of neutron activation will take place in some components of the reactor and precautions in plant design and hygiene will have to be taken to prevent the creation and escape of biologically hazardous nuclei. Fourth, tritium will be present and will be processed in large quantities in a fusion reactor. Although tritium is a biological hazard, it is less dangerous than most radioactive species because of its rapid dissipation in the atmosphere and because of the low penetrating power of its radiation.

Fifth, a fusion reactor operating on the DT cycle will contain large quantities of fused lithium in the form of a layer of thickness 1 m around the reaction space. Lithium is a chemical fire hazard in the same way as the sodium in fission reactors.

Even a technically expert saboteur in a fusion power plant could do no more than stop it and, in the case of a DT cycle plant, set off a lithium fire and release tritium in quantities that would not be catastrophic but would depend on the construction of the plant.

Fission, with all its disadvantages, could be improved by a wise siting policy. This would be to install plants on remote sites with their own fuel element processing and fission product disposal (and preferably, for its thermodynamic advantage, with cold water cooling). Fuels would enter such a plant but no radioactive or fissionable material would leave it. This reduces the hazard of fissionable material dispersal by theft and is also advantageous because of the low density of the surrounding population. Such plants would naturally form the power input points of a nationwide power grid. Many advanced countries today have such grids and, although a linking of privately owned power companies is taking place in the United States, the formation of a national power grid is somewhat overdue.

The objections made to such an obviously logical course by

power plant operators have been, first, the high losses in long distance bulk power transmission, and, second, the high right-of-way costs of bringing transmission lines into cities. Both these arguments are valid if only on account of many years of neglect of development in the field of power generation and transmission. Overhead high voltage d.c. transmission as developed in Sweden is being tried in the United States (Bonneville to Los Angeles). The transmission of high voltages underground, ultimately by superconducting lines, is obviously the way to bring power into cities, both from the point of view of the amenities and the cost of right-of-way.

According to a recent *Fission Power Reactor Review*¹², there are more than 300 large fission reactors in existence or being built. Thus, fission power not only exists but is in a flourishing state. The disadvantages fission has will have to be lived with and it is not too late to do better. Fusion does have advantages over fission, and, in my opinion, will ultimately supersede it. The critical question is—when might this takeover occur?

Time Scales

December 1942 is the birth date of fission, when the first pile went critical. Two years later, under the wartime thrust of the Manhattan project there came into existence a 1,000 MW (thermal) fission reactor (Hanford). It is less easy to put a firm date on the achievement of economic fission power—1965 might be correct for the United States, but it came earlier in Britain where power costs are higher and competitiveness easier to achieve. Accordingly, the development time of fission power from birth to usage will be taken as twenty years.

The term feasibility has been widely used in the search for a comparable birth date for fusion. The energy balance for a fusion reactor plasma is determined first by the ion temperature T_i , which must be above a certain minimum value— ~ 10 keV for most reactor geometries using the DT cycle but as high as 100 keV for the special case of the mirror machine with direct conversion. The second determining factor is the Lawson energy balance criterion $n\tau$, where n is the plasma density and τ the plasma confinement time. These two parameters can evidently be traded off against one another and so there can be a considerable range of parameters capable of satisfying the $n\tau$ reactor criterion, that is low density and quasi-steady state (with $n \sim 10^{15}$ ions cm^{-3} , $\tau \sim 1$ s) up to high

density, pulsed state (with $n \sim 10^{23}$ ion cm^{-3} and $\tau \sim 10^{-8}$ s). The energy break-even point for a DT reaction is at $n\tau \sim 10^{14}$ ion cm^{-3} s, and the achievement of this has been called feasibility. For a reactor, one would need to achieve $n\tau \sim 10^{15}$, say, which would give an output of about ten times the input.

In Fig. 1, these quantities T_i and $n\tau$ are plotted on an achievement chart for the plasmas at present obtained in reactor-oriented experiments. (The laser-heated DT pellet approach (for example) is, however, too recent for significant plasma achievements to have been made.) Although at this early stage it is probably not significant, the θ -pinch is at present leading the race to reach feasibility by a factor of 2 in T_i and 100 in $n\tau$. But an increase in $n\tau$ by a factor of 10^3 is still necessary before reactor status can be achieved. For the special case of mirror machines with direct conversion, the reactor target area is different, with a much higher T_i , > 100 keV, but a lower $n\tau$, $\sim 10^{14}$.

For the benefit of those unaware that a controlled thermonuclear reaction had been achieved at all in the laboratory, it should be noted that the Scylla 4 θ -pinch plasma can generate (DT) a gross output of 100 kW of thermonuclear heat for a few μs . It is much too early to predict which of the contenders will reach the target first—the next steps forward at Tokamak (T-10) and at the θ -pinch (Scyllac) will, hopefully, advance $n\tau$ by a factor of 50.

The rate of achievement will depend in some less than linear way on the degree of financial support: a reasonable extrapolation at the current rate of progress suggests that feasibility might be reached by several routes in, say, 1980. Such an achievement would in no sense be a reactor; it would merely be a plasma which put out as much potentially recoverable energy as had been put into it—the engineering complexities, walls, heat exchangers would still lie ahead.

Starting from 1980 as the birth date for fusion, we now have to estimate the development time. It is hard to imagine anything simpler than a fission chain reacting pile, but a fusion reactor is bound to be much more complicated. It took twenty years to develop fission, but then there was no previous experience with radioactivity on a large scale.

I shall take the two factors of increased fusion complexity and increased engineering experience with radioactivity as mutually compensating, and the development period for fusion to be the same as for fission—twenty years. Competitive fusion can, therefore, be expected by the year 2000.

Fusion could have undoubted advantages over fission socially but from the point of view of ecology and pollution there is actually very little to choose between them, and both are greatly superior to fossil plants (especially those fired by coal).

Perhaps because of a confusion between the social and the ecological roles, fission has come under widespread attack from ecologists and anti-pollutionists recently, whereas fusion is being held up as the ideal. This use of fusion as a stick with which to beat fission seems also to have created a political polarization which is ill-founded technically and, if allowed to continue, may work to the disadvantage of fission, fusion and mankind generally.

For a fusion reactor to produce power, it must achieve the requisite ion temperature (~ 10 keV for DT, ~ 50 keV for DD) for a sufficient $n\tau$ for the energy output from the thermonuclear reaction ($\propto \epsilon n^2 \tau Q \sigma v \text{ cm}^{-3}$) to exceed the input energy investment in heating the plasma ($\propto n T \text{ cm}^{-3}$); Q is the energy yield of the nuclear reaction, σv is the nuclear reaction cross section averaged over the Maxwell distribution of velocities in the plasma and ϵ is the Carnot efficiency of the heat-electricity conversion. The aim of the magnetic confining system (bottle), if there is one, is to be as economical as possible both in its construction and energy running costs. This implies that the magnetic field B shall confine the maximum possible plasma pressure, or that the ratio of plasma pressure nkT to magnetic field pressure $B^2/8\pi$ shall be as near to unity as possible. A plasma confined by an external magnetic field has the fraction $(1-\beta)^{1/2}$ of that field inside. Some characteristic

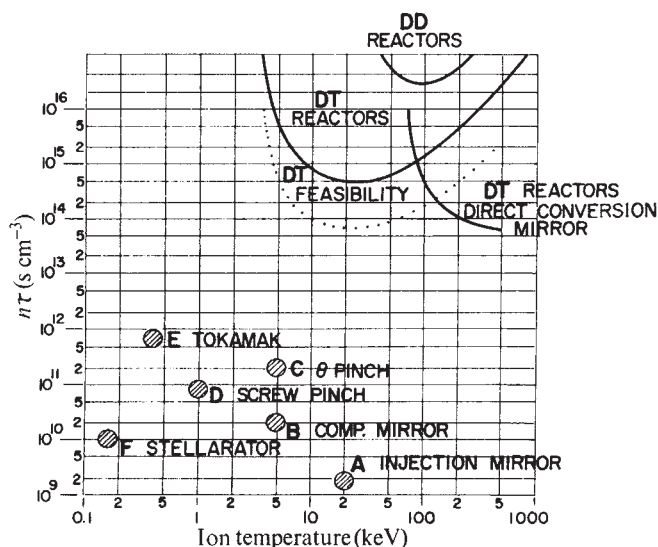


Fig. 1 Status diagram of fusion approaches to power reactors. A, Injection mirror machine Phoenix (Culham, UK); B, compression mirror machine 2X (Livermore, USA); C, θ -pinch Scylla 4 (Los Alamos, USA), also ISAR I (Garching, W. Germany); D, screw pinch ISAR IV (Garching, W. Germany); E, Tokamak T-3 (Moscow, USSR) since duplicated by S.T. (Princeton, USA); F, Stellarator-C (Princeton, USA).

Table 1 Characteristic Parameter Values for a Possible DT Reactor Plasma

Ion temperature	T_i	10 keV ($\equiv 114 \times 10^6$ K)
Electron temperature	T_e	10 keV
Ion density	n	10^{15} ion cm^{-3}
Electron density	n	10^{15} electron cm^{-3}
Confinement time	τ	1 s
Plasma pressure	$2nT$	3.2×10^7 dyne cm^{-2} (≈ 32 atm.)
Magnetic field	$\beta = 1$	21 kgauss
	$\beta = 0.01$	210 kgauss
Velocity of a 10 keV deuterium ion		10^8 cm s^{-1}

parameter values for a possible DT reactor plasma are given in Table 1.

The last parameter is an indicator of the rapidity with which the plasma moves when free to do so or with which it escapes when an instability defeats the magnetic confinement.

Tokamak

Tokamak is a large toroidal system with thick copper walls and a toroidal winding encircling a magnetic core. A large current is induced in the plasma encircling the core, which heats the plasma resistively, and a large longitudinal magnetic field from the toroidal winding stabilizes the z pinch-like configuration; additional stabilization is provided by the conducting wall. The approach was devised early in the days of controlled thermonuclear research at the Kurchatov Institute, Moscow, by Artsimovich and his co-workers. From the 1950s onwards, a series of models of increasing size were produced but they aroused little interest among the main stream of plasma physicists. The breakthrough occurred in 1969 when an ion temperature of 0.4 keV was achieved in the T-3 machine; the pulse duration was 20 ms (ref. 5). The density is in the region 2 to 5×10^{13} , giving a value of $n\tau$ of 10^{12} , the largest yet achieved anywhere. The result was surprising, according to conventional theory, because such temperatures are not accessible by Joule heating with classical plasma conductivity. The resistivity of the Tokamak turned out to be 5 to 10 times the classical value and this has been explained in terms of a

reflects great credit on Artsimovich and co-workers. It is far from clear how the Tokamak can be extrapolated to a reactor, as the Russian physicists readily concede. For one thing the present method of Joule heating will not continue from 0.4 keV to the required 10 keV even with anomalous resistivity although supplementary heating processes, such as ion cyclotron heating, have been proposed. It is hard to imagine that with the present low value of β ($\sim 10^{-3}$) a reactor could be economic, so some method of confining stably more plasma and exceeding the Kruskal-Shafranov limit seems to be needed. The plasma density can also be increased by reducing the torus aspect ratio—making it fat. At the most recent Fusion International Conference at Madison, Wisconsin, in June 1971, a most ingenious proposal was made for self-driving of the longitudinal plasma current by the outward diffusion of new fuel injected into the axis. This may greatly reduce magnetic core requirements, and even offers the possibility of a steady state reactor. Other basic problems of extrapolating Tokamak into a reactor arise from the presence of the multiplying blanket and from synchrotron radiation by the hot electrons. The 1 m thick blanket must be interposed between the heavy torus windings—which are too thick to transmit neutrons—and the plasma. The presence of the 1 m standoff weakens the stabilizing effect of the wall so other stabilizing means are being sought; a scale size for a reactor in the 10,000 MW_e range is thus established. Fig. 2 is a Russian schematic drawing of a Tokamak power reactor showing the scale and general appearance; the heat exchangers, turbine, injection and processing auxiliaries are not shown.

The synchrotron radiation flux from the electrons of the plasma in a magnetic field has been estimated theoretically but the flux has not yet been observed experimentally. The radiation is an individual process and therefore emitted at a rate proportional to the density—by contrast with the thermonuclear fusion reaction itself which proceeds at a rate proportional to the square of the density. Consequently high densities favour the desired fusion over the undesired radiation. In a reactor situation, synchrotron radiation increases as β^{-3} and becomes the dominant process at values of β less than about 0.01. The subject of synchrotron radiation is theoretically difficult and tends to have been treated superficially in reactor studies. The radiation will have a wavelength in the region 0.01 cm and this

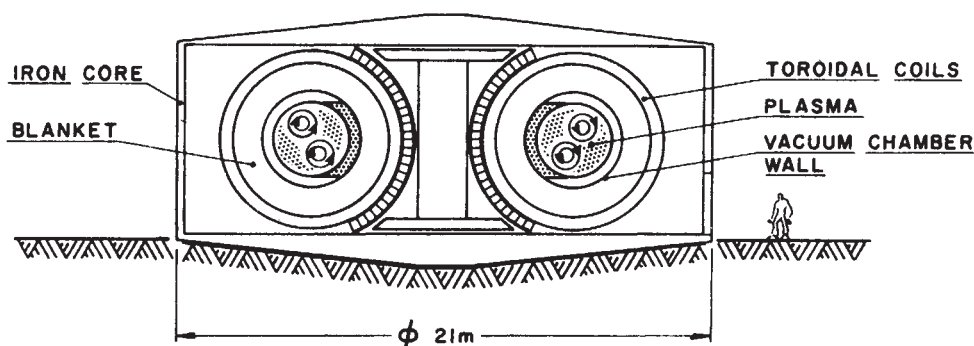


Fig. 2 Tokamak reactor. Fuel, D+T; thermal power, 5 GW; electrical power, 2 GW; major plasma diameter, 14 m; maximum magnetic field, 100 kgauss; plasma density, 3×10^{14} cm^{-3} ; confinement time, 0.7 s; $\beta = 0.3$. Two of many ion orbits are shown in the plasma region. (After Golovin, "Energy 70", Las Vegas, 1970.)

plasma turbulence. There is a critical limit to the current that can be passed through the plasma (in terms of the longitudinal magnetic field), which is such that the spiral executed by the resultant magnetic field does not close on itself in one circuit of the torus (Kruskal-Shafranov limit). This implies a very low value of β for the plasma. A visit by Artsimovich to the United States convinced physicists there of the validity of the results—the first time thermonuclear temperatures have been achieved in a slow, low- β system. This produced a somewhat surprising landslide toward Tokamaks in the United States with the result that five machines of differing designs have been authorized (at Oak Ridge National Laboratory, Princeton Plasma Physics Laboratory, Austin, Texas, MIT and Gulf-General Atomic at La Jolla). In the meantime, the construction of a much larger Tokamak, T-10, has commenced in the USSR.

The Tokamak is a very real experimental advance which

is easily reflected by metal mirrors with an efficiency of 99%. By completely surrounding the plasma with such a reflector, the synchrotron losses can be greatly reduced though the radiation intensity inside the shield rises above its previous value. The problem is that, quite apart from the effect that the enhanced radiation will have on the stability of the plasma it is certain that engineering requirements pumping ports and so on, will make it very difficult to achieve 99% perfection in the reflecting enclosure. A theoretical calculation for the Tokamak indicates that synchrotron radiation loss would be a significant factor in the energy balance for a Tokamak without reflectors⁶.

Direct Conversion

Most (~ 14 MeV) of the total Q of 18 MeV associated with the DT reactions appears in the form of neutron energy. This

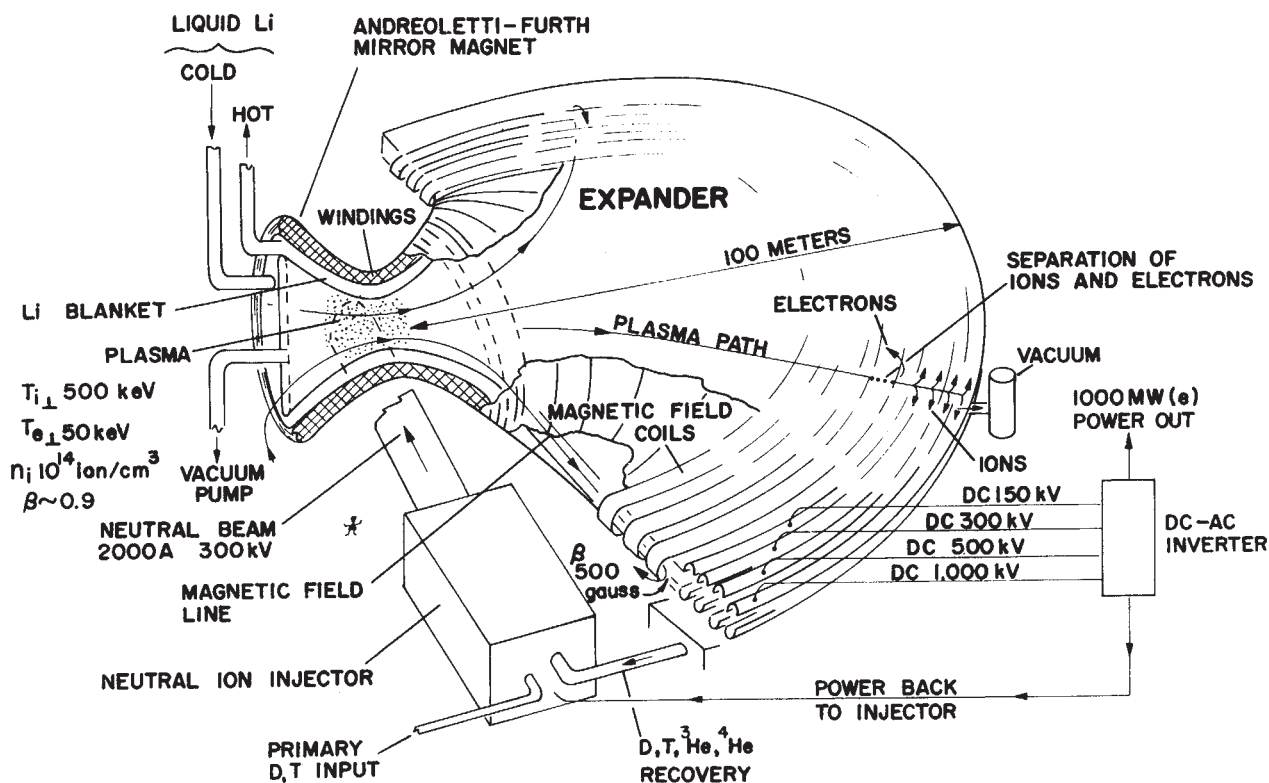


Fig. 3 Direct conversion mirror fusion reactor.

energy can be utilized only by depositing it in a target as heat and passing the heat through a thermal cycle (for example, a Carnot cycle). The efficiency of a Carnot cycle between upper temperature T_1 and lower temperature T_2 is $(T_1 - T_2)/T_1$ and, for practical upper temperature ~ 750 K and lower temperatures 373 K, this value is about 0.3 to 0.4. This efficiency appears in the Lawson break-even equation for $n\tau$ as $(1 - \epsilon)/\epsilon$ or, with $\epsilon = 0.3$, a factor ~ 2.3 . In the DD reaction, only 38% of the Q of ~ 4 MeV appears in the neutron, so that most of it is evident in the form of α particles, protons and ^3He nuclei. In the case of the D^3He reaction, all the energy of the reaction is emitted as charged particles. Now although the neutron energy can only be converted into power by the low efficiency thermal cycle described previously, charged particle energy could, in principle, be converted much more efficiently either by allowing the plasma of charged particles to do work on a magnetic field directly or by separating the charged components of the plasma and discharging them into conductors, that is, direct conversion. In either case, the upper temperature of the Carnot cycle becomes, in effect, the plasma temperature $\sim 10^8$ K. Taking T_1 as 10^8 K, this gives a value of ϵ of 0.999, so $(1 - \epsilon)/\epsilon \sim 10^{-3}$; this brings the Lawson criterion $n\tau$ down from its previous value by a factor of 2,300. According to the calculation for this ideal, DD fusion with direct conversion would be easier, in terms of $n\tau$, than DT fusion without it.

Post⁷ has made the ingenious suggestion that the exhaust plasma from a mirror machine should be expanded by a large factor. This has the effect of converting the initially random motion of the plasma into a quasi-ordered beam, that is, an isentropic expansion. The beam of plasma is separated into its ion and electron components by magnetic deflexion, and the charges in the two components collected in stages to provide high voltage d.c. power. The expander is of considerable size (Fig. 3) but the elimination of the tiresome and inefficient heat exchanger-boiler-turbine combination characteristic of other fusion reactors, and the achievement of a power plant with no moving parts, would be more than worth while.

Not only does the mirror machine have to operate at a much higher ion temperature ($\gtrsim 100$ keV) than other systems but the

electron temperature has to stay low ($\lesssim 30$ keV) and decoupled from the ions; this is a thermodynamic disequilibrium which invites instability. If instability should occur, then the electron temperature will rise, bringing trouble from increased synchrotron radiation. Furthermore, in mirror machine reactors the fraction of plasma burn-up during one passage of the plasma through the machine is small so a large amount of energy (compared with the useful output energy) has to be recirculated—that is, plasma is reinjected into the machine. This means that the achievement of a positive power balance depends critically on a very high efficiency, $> 90\%$, in the stages—output beam-to-electricity converter and electricity-to-input-ion beam accelerator—through which the large recirculating power passes. These values are based on a classical mirror confinement time. There is evidence that the loss out of the mirrors is 10 to 15 times faster than this because of the effect of theoretically predicted instability. If this instability cannot be overcome, the stage efficiency requirement will become even more stringent—to the extent, possibly, of being unrealizable.

It is a most interesting idea whose application may extend to the thermonuclearly more efficient high β , open-ended, linear Scylla-type reactor. This is already capable of yielding a positive power balance using a DT fuel at 10 keV temperature and the usual low 0.3 efficiency for ϵ . If direct conversion, with an efficiency $\epsilon = 0.9$, were applied, $n\tau$ could be lowered by a factor of ~ 20 to 30 —which might yield a positive power balance even with DD. The blanket could then be omitted and the whole system made much simpler.

Scylla-Scyllac θ Pinch

Plasma in a long tube is subjected to a rapidly rising longitudinal magnetic field. This has the effect of first shocking the plasma, and then adiabatically compressing it. The elegant aspect of this approach is that the heating is applied directly to the ions, where it is needed; the heating is not resistive (which would heat the electrons first) and does not fail when the temperature is very high and the plasma is exceedingly con-

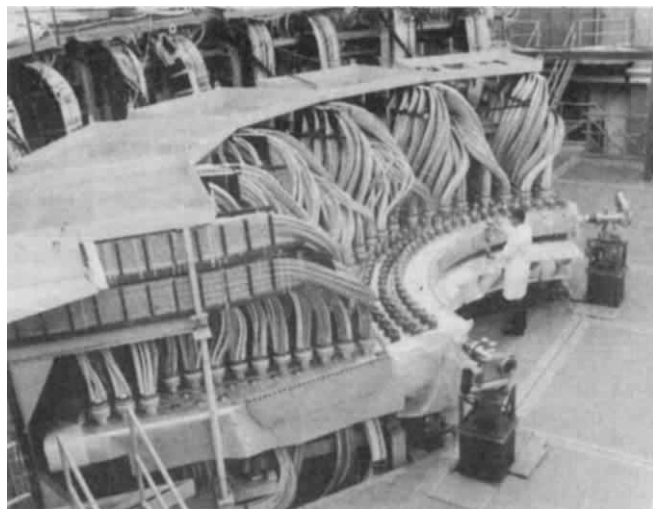


Fig. 4 Completed 120° arc of the Los Alamos Scyllac toroidal θ pinch.

ductive. Unlike many other approaches, the hotter the plasma gets the better behaved it has been found experimentally to become. This device in its early form (Scylla I) produced the first laboratory thermonuclear reaction in 1957, although it was not until 1961—after many experiments—that the thermonuclear character was established beyond doubt. Achievements with Scylla IV are T_{ion} , 5 keV; n , 5×10^{16} ion cm^{-3} ; τ , 5 μs ; $n\tau$, 2.5×10^{11} ion cm^{-3} s; (β (max) ~ 0.9 ; β (av) ~ 0.3).

Although the Scylla θ pinch was originally conceived⁸ only as an experiment to make thermonuclear plasma, it was later shown⁹ that, in principle, it could become a reactor. The idea was to make the compression coil thin enough to be essentially transparent to the 14 MeV neutrons. This allows the T regenerating blanket of thickness 1 m, which is also the flowing

Li-Be heat removing fluid, to be outside the coil.

The temperature achieved is already within a factor of two of the target temperature for a reactor, and there is no obvious difficulty in raising it further. At present, the confinement time $\sim 5 \mu\text{s}$ is determined by free escape of the plasma from the ends of the compression coil. There are two ways to extend this time. One is to close the compression coil on itself to form a torus. This distortion introduces field gradients, disequilibrium, and calls for entirely new countermeasures to give stability and to hold the plasma in the centre of the tube. It is not yet known whether stability will be obtainable statically or whether dynamic or servo methods may have to be used. In its toroidal form, this device is known as Scyllac (Scylla-closed), and it is under construction at Los Alamos (Fig. 4). It will be some years before it is completed and, ideally, it is expected to increase $n\tau$ by a factor of up to 50 from the present value of 2.5×10^{11} .

Alternatively, it is possible to increase confinement time by lengthening the present straight system. This certainly has the merit of simplicity and the magnetic field is uniform. To reach a value of $n\tau$ appropriate to a reactor by this method alone would, however, require a length of several km. But it may be possible to restrict the loss from the ends and a roughening procedure in the magnetic field has given some theoretical support for optimism, in essence giving the plasma flow Knudsen¹⁰ characteristics. A loss reduction by a factor x reduces the reactor length by the same factor. Electron synchrotron radiation is likely to be much less of a problem in θ pinches because they are high n , high β devices. The straight θ pinch reactor has a simple geometry (Fig. 5) and involves the least extrapolation beyond present plasma physical knowledge and experience. The ends form a natural way of disposing of the plasma, and may even provide extra power by direct conversion or by a pulsed magnetohydrodynamic generator.

The great length of the reactor in its unstoppered form makes it impractical and, above all, the engineering problem of supplying economically the enormous pulse power (proportional to the length) is unresolved. Condensers are also too

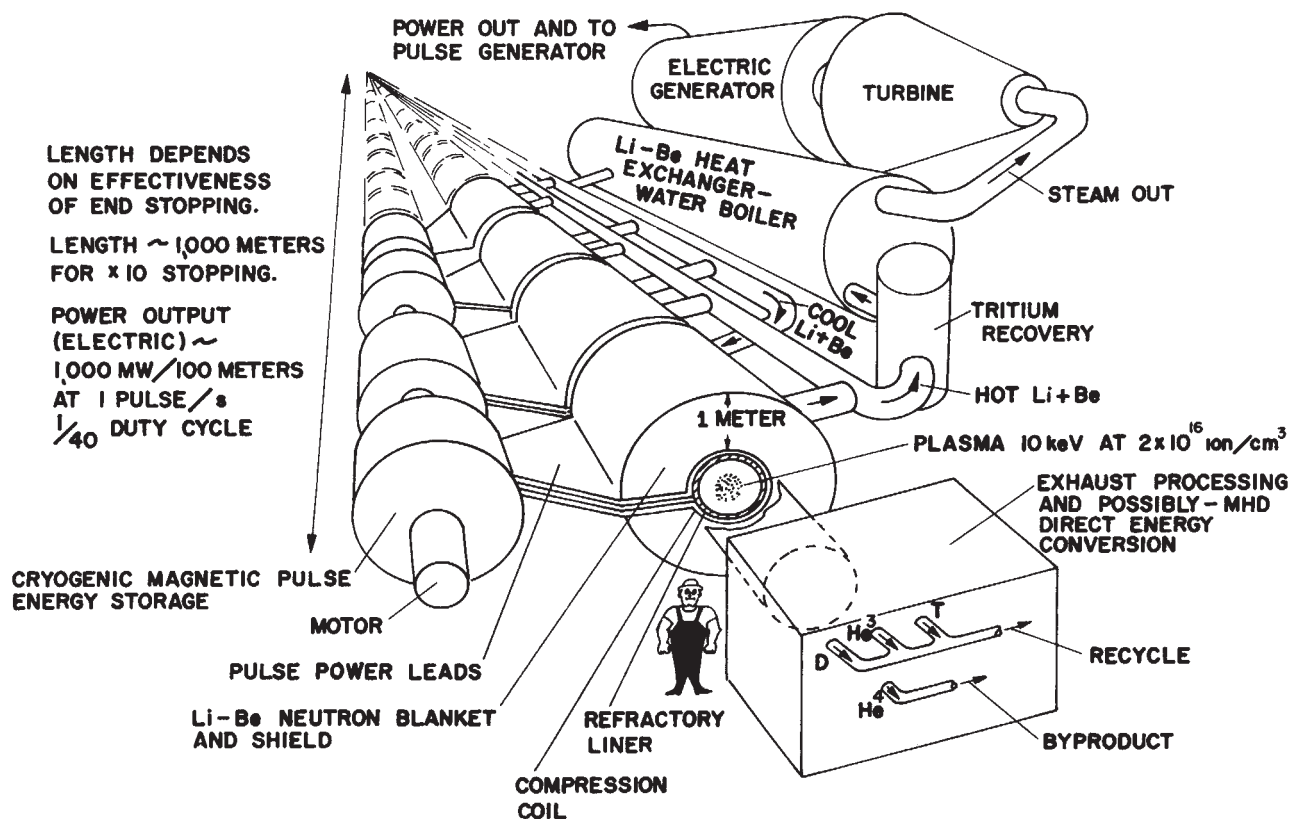


Fig. 5 Scylla linear θ -pinch fusion reactor (schematic).

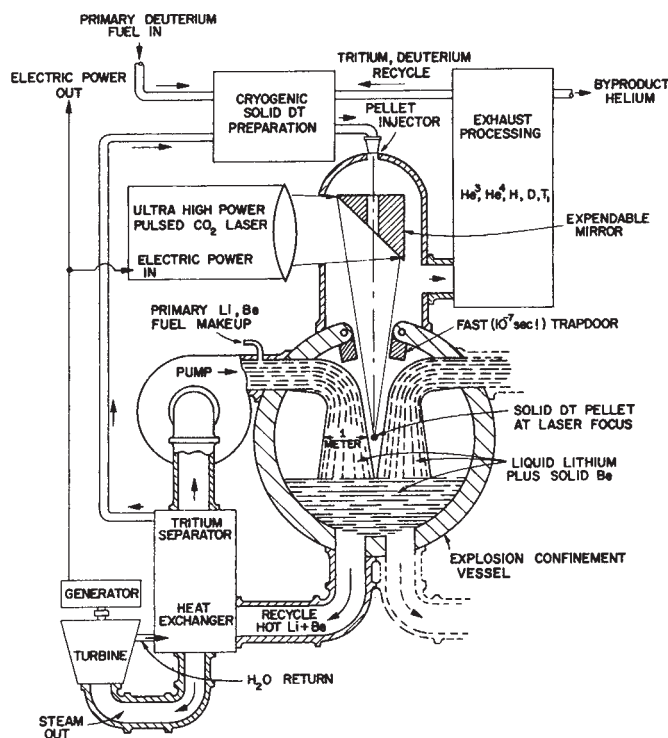


Fig. 6 Conceptual (1969) laser DT pellet reactor.

expensive but rotating superconducting generators¹¹ have been proposed.

The toroidal Scyllac is more radical in concept and more liable to instability but has lower pulse power requirements. The serious problem of feeding plasma in and removing it without damage to the inner wall has not yet been tackled.

Laser Heated DT Pellet

The density of solid DT at 4 K is 6×10^{22} atom cm^{-3} . If a pellet of radius r could be heated instantaneously to 10 keV, a strong thermonuclear reaction during the rapid expansion (velocity $\sim 10^8$ cm s^{-1}) would take place. The effective confinement time will be $\sim r/10^8$ s.

From Fig. 1, it can be deduced that r would have to be about 1 mm to reach reactor status. Expressed alternatively, the input energy is proportional to the volume r^3 , but the thermonuclear output energy is proportional to this volume and the time (one more factor of the radius) or r^4 . Thus there has to be a value of the radius such that the output exceeds the input. According to elementary plasma theory, at the density of 6×10^{22} , radiation from known lasers would be below the plasma cut-off frequency for that density and would be reflected. It is far from clear whether this holds true at the radiation intensities existing at a laser focus. Further, a pre-preparation of the pellet surface density can be envisaged, which would increase the laser energy absorption at the surface. At first, in the mid-1960s, it seemed to many of us that such ideas were hopeless for power production, for the pumping efficiency of, say, Nd glass lasers was $<1\%$ —which imposed a hundred-fold adverse energy factor at the outset. More recently, however, the CO_2 laser has achieved an efficiency $>20\%$ (theoretical limit $\sim 40\%$), which might just be acceptable.

The reactor that might develop from these ideas (Fig. 6) is the model of simplicity—no magnetic fields, no stability. In fact instability is an advantage, for it may help the heating. The pellet is dropped into a boiler which is strong enough to withstand the explosion and the inner walls of which consist of a rapidly flowing 1 m layer of liquid lithium, which acts as the tritium regenerator and carries off the heat to the exchanger. There should be no trouble about heating of the wall, a sensitive point in all other fusion reactors, as it is constantly renewed.

The energy input and output are sensitive functions of the pellet radius at break-even. If the laser energy input for positive energy balance turns out to be, say, 10^5 J—which is some distance beyond present laser practice but imaginable—and the energy (thermal) output is, say, 10^7 J—equivalent to a few pounds of TNT which could easily be confined, even repetitively in a boiler of reasonable dimensions—the reactor will be very attractive. But an increase in the break-even radius of, say, a factor of ten would increase these figures by a factor of 10^3 ; this would place the system far from reality and would necessitate a boiler which could withstand the equivalent of several tons of TNT exploding repetitively—it would be beyond present engineering practice. Clearly it is very advantageous to increase n because this diminishes the τ for break-even in inverse proportion, which in turn brings down the required input energy by the same proportion to the third power. Any way of slowing down the expansion rate pays off equally handsomely.

It should be obvious that there are very rewarding directions for the application of ingenuity to this problem. And there is even an alternative possibility to the laser for the heating source—a pinched-down beam of relativistic electrons.

There are two other interesting problems. The laser radiation must be admitted into the boiler through some aperture and, clearly, the explosion must not destroy the optics. There is very little time between the admission of the radiation and the impact of the explosion shock-wave on reaching the walls, so the manipulation of a trapdoor would be very difficult. Thin metallized plastic expendable mirrors spring to mind, however. Finally, factors of reactor economics common to all fusion reactors show up with great clarity in this case. One kWh (electric), $= 3.6$ MJ, can be generated currently for 0.3 to 0.5 cents and therefore that is all that can be spent per shot (for the 10^7 J case above) to cover the laser pumping, plant, salaries, processing, DT and cryogenics. It will obviously have to be a very efficient operation. It is too early to guess how the DT pellet reactor will fare in the fusion reactor race. Superficially it looks very good—but this may be because it is also the newest. In France, this approach is being studied at Limeil and in the United States at Livermore, Los Alamos, and Oak Ridge. At the Lebedev Institute, Moscow, they are also thinking along similar lines.

All fusion reactor approaches have unsolved problems and their problems differ greatly in kind. Nonetheless, it seems to me very likely that out of the diversity, one at least will succeed. Nuclear energy is certainly essential for the future of mankind and, although fission is solving some of the energy problems, fusion will in many ways be far more satisfactory. But it will probably not become competitive for thirty years.

This work was performed under the auspices of the US Atomic Energy Commission.

- 1 Brown, A., Bonner, J., and Weir, J., *The Next Hundred Years* (The Viking Press, 1957).
- 2 Weinberg, A. M., *Physics Today*, 18 (1959).
- 3 Tuck, J. L., in *Cosmology, Fusion, and Other Matters* (Colorado Associated University Press, in the press) and *Los Alamos Scientific Laboratory Preprint LADC-9519*.
- 4 Robinson, E. S., et al., *Los Alamos Scientific Laboratory Report LA-4547* (1971).
- 5 Artsimovich, L., et al., *Proceedings of the Third Intern. Atoms for Peace Conference, Novosibirsk, 1968*, 1, 157 (IAEA, Vienna, 1969).
- 6 Rosenbluth, M. N., *Nuclear Fusion*, 10, 341 (1970).
- 7 Post, R. F., *University of California, Livermore Radiation Laboratory Report UCRL-71753* (1969).
- 8 Boyer, K., Elmore, W. E., Little, E. M., Quinn, W. E., and Tuck, J. L., *Phys. Rev.*, 119, 857 (1960).
- 9 Quinn, W. E., Ribe, F. L., Riesenfeld, W. B., Sawyer, G. A., and Tuck, J. L., *Los Alamos Scientific Laboratory Report LA-3294-MS* (1965).
- 10 Tuck, J. L., *Proc. Third Internat. Conf., Novosibirsk, 1968*, 2, 595 (IAEA, Vienna, 1969).
- 11 Smith, P. F., *Synchrotron Power Supplies using Superconductive Energy Storage*; *Rutherford (UK) Laboratory Report* (1967).
- 12 *Nuclear Engineering International*, 16, 176/177 (1971).

Acetylcholine Receptors in Muscle Fibres

R. MILEDI & L. T. POTTER

Department of Biophysics, University College, London

There are calculated to be approximately 10^9 acetylcholine receptors in a frog muscle fibre. This has important implications for theories concerning the nerve to muscle transmission mechanism.

THE transmission of impulses from nerve to skeletal muscle fibres of vertebrates is mediated by the release of a transmitter—acetylcholine (ACh)—from the motor nerve endings. After diffusing across a narrow synaptic cleft, the ACh molecules react with a specific “receptor” in the muscle fibre membrane, and consequently the ionic permeability of the membrane is greatly increased. While the process of transmitter release has been relatively well studied¹, our knowledge of ACh-receptors is, so far, fragmentary.

Intracellular recording and micro-ionophoretic application of ACh have established that the ACh-sensitivity of normal muscle fibres is maximal in the region of neuromuscular contact and falls sharply therefrom². Such experiments demonstrated the pattern of distribution of the ACh-receptors but not their number, which can apparently now be obtained with the use of snake toxins.

It has long been known that several snake venoms have a curare-like action³. Chang⁴ showed clearly that the venom from a Formosan snake (*Bungarus multicinctus*) abolished neuromuscular transmission by blocking the action of ACh, apparently in an irreversible manner. Chang and Lee⁵, and Lee, Tseng and Chiu⁶, subsequently established that a subfraction of this venom blocked neuromuscular transmission by combining with a site on or near ACh-receptors. We have confirmed and extended their work.

Twelve pure proteins were isolated from the dried venom of *B. multicinctus* (details to be published elsewhere). The most prevalent of these (20–25% of the protein of the venom) is a basic protein with a molecular weight of 8,000; it probably corresponds to the “ α -bungarotoxin” of Chang and Lee⁵, and was used for our experiments.

ACh-receptors in Normal Muscles

When α -bungarotoxin (α -BuTX) was added to Ringer solution bathing frog or rat muscles, neuromuscular transmission was blocked. Nerve impulses continued to invade the nerve terminals and release ACh, but the action of ACh on the postsynaptic membrane was progressively reduced. Fig. 1 illustrates the effect of 10^{-6} g/ml. of α -BuTX on the amplitude of the end-plate potential (e.p.p.) which can be taken as an index of transmitter action on the muscle fibre membrane. At zero time the bath was changed to one containing the toxin

and, when the microelectrode was reinserted into the fibre a few minutes later, the e.p.p. was already reduced. Subsequently the e.p.p. amplitude continued to fall, approximately exponentially, with a half time in this case of about 2.25 min. The sensitivity of the end-plate to ionophoretic or bath application of ACh is concomitantly reduced.

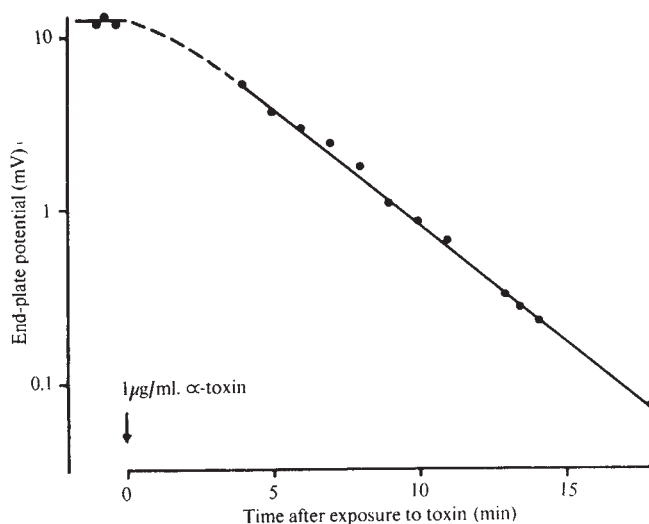


Fig. 1 Effect of α -BuTX on the amplitude of end-plate potentials from a surface end-plate in a frog sartorius muscle. Each point is the average of twelve potentials.

The time-course of blockade was concentration-dependent, and also varied appreciably from fibre to fibre within a muscle. End-plates deep within the muscle took longer to block than surface end-plates, but with 10^{-6} g/ml. α -BuTX all end-plates of frog sartorius muscles ceased to transmit impulses in 1–2 h. This variability is very likely due to limitations in the access of the toxin to the end-plates, either because of diffusion barriers or because the toxin reacts at other sites on its way to the end-plates. In some experiments α -BuTX was applied ionophoretically. This makes it possible to block only a small part of an end-plate, and the rate of block is then much faster than shown in Fig. 1.

Several muscles were exposed to 10^{-5} g/ml. of α -BuTX for about 2 h, and were then washed for several hours at room temperature, with no sign of recovery of ACh-sensitivity. Washing was then continued at 3–4° C and even more than 8 days later the muscle fibres remained excitable but there was still no recovery of either neuromuscular transmission or sensitivity

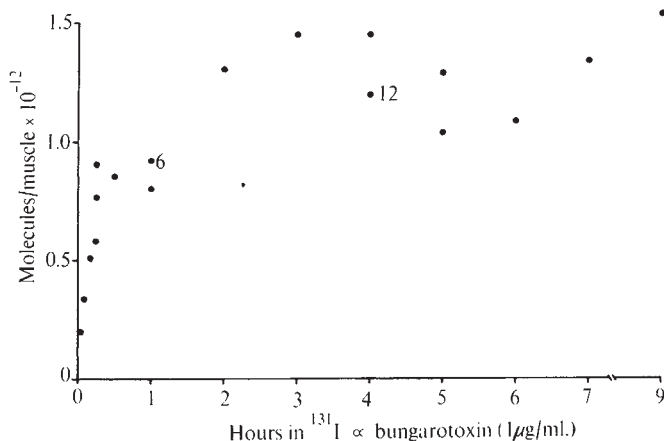


Fig. 2 Saturation of toxin-binding sites of frog sartorius muscles. Muscles were exposed to ^{131}I - α -BuTX for the periods indicated; then washed and assayed for radio-iodine (see text). Points represent single muscles or mean values of groups of muscles the number of which is shown.

to acetylcholine, which shows that blockage of ACh-receptors by α -BuTX is, for practical purposes, irreversible.

To study its binding to cholinceptive tissues α -BuTX was labelled⁷ with ^{131}I . Such labelling consistently gave an initial specific activity (of 23,000 Ci/mol) with no observable loss in the ACh-receptor blocking activity of the toxin. Muscles were exposed to the labelled toxin at 20° C for various times and then washed free of unbound toxin by repeated bath changes for about 24 h at 4° C. The tissue was then dissolved in an alkaline solvent ('Soluene', Packard), and the amount of ^{131}I was determined by liquid scintillation spectrometry. Corrections for isotopic decay were made by measuring the activity of the stock solution of ^{131}I α -BuTX at the same time as a series of muscle samples.

The time-course of toxin-binding to frog sartorius muscles is shown in Fig. 2. Binding increased during the first 2 h at a rate comparable with that of neuromuscular blockade, and then reached a plateau level of 1.2×10^{12} molecules of toxin per muscle (mean of twenty values obtained after 2 h). Similar experiments with diaphragms of rats and mice gave respective values of 4.7×10^{11} and 1.6×10^{11} toxin-binding sites per hemidiaphragm (means of twenty-eight and six respectively).

If the amount of bound toxin is to be used to estimate the number of ACh-receptors or to mark them for subsequent isolation, it is important to determine the specificity of the binding. One measure of the specificity of toxin-binding to receptors is its localization on the muscle fibres. Using autoradiography, Lee, Tseng and Chiu⁶ have shown that in rat most of the label is in the end-plate region. To obtain a more quantitative estimate of the localization of binding, we have divided muscles into portions, some of which were practically free of end-plates. In the frog sartorius, "end-plate free" portions (amounting to about 20% of the wet weight of the muscles) had an average of 2% of the total counts (mean of twenty-four muscles); and even some of this binding is probably still associated with the ACh-receptors which are known to exist in "end-plate-free" regions^{8,9}. In the rat diaphragm end-plate-free portions accounted for five-sixths of the muscle and had about 10–15% of the total radioactivity. All this shows that non-specific binding of the toxin is not a large complicating factor.

As most of the toxin is bound at end-plate regions, the plateau level of binding (Fig. 2) may be used to estimate the number of toxin-receptive sites per end-plate. In the frog sartorius muscle, which has about 500 muscle fibres and two to four end-plates per fibre^{10,11}, the number of toxin-receptive sites per end-plate is about 10^9 . The corresponding number for

the rat hemidiaphragm which has about 10^4 singly-innervated fibres¹² is about 4.7×10^7 sites per end-plate. Assuming the same number of fibres in the mouse hemidiaphragm gives a value of 1.6×10^7 sites. The last figure is several times larger than the number of curarine-binding sites found by Waser¹³, perhaps partly because curarine, a bisquaternary ammonium compound, blocks only half of the toxin-binding sites (see below). In this context, it is interesting that Changeux, Kasai and Lee¹⁴ have found roughly twice as many binding sites for α -BuTX as for decamethonium (another bisquaternary amine) in the electric tissue of eels.

ACh-receptors in Denervated Muscle

After denervation the entire muscle fibre membrane develops ACh-receptors with properties resembling in most respects those of the junctional receptors^{8,11,15}. They are, however, less sensitive to curare¹⁶, and it was therefore important to find if the new receptors are also blocked by α -BuTX. The ACh-sensitivity of muscle fibres was measured¹¹, some 6 mm away from the end-plates, in a muscle which had been denervated for 28 days. After 3 h in α -BuTX (10^{-6} g/ml.) the ACh-sensitivity had fallen by more than 1,000 times, a clear indication that α -BuTX also blocks the newly developed ACh-receptors. Accordingly, the amount of bound toxin was also increased after denervation. The number of toxin sites per muscle increases progressively (Fig. 3), reaches a peak and then starts to decrease, presumably because the muscle fibres atrophy and decrease in size and number on account of the prolonged denervation.

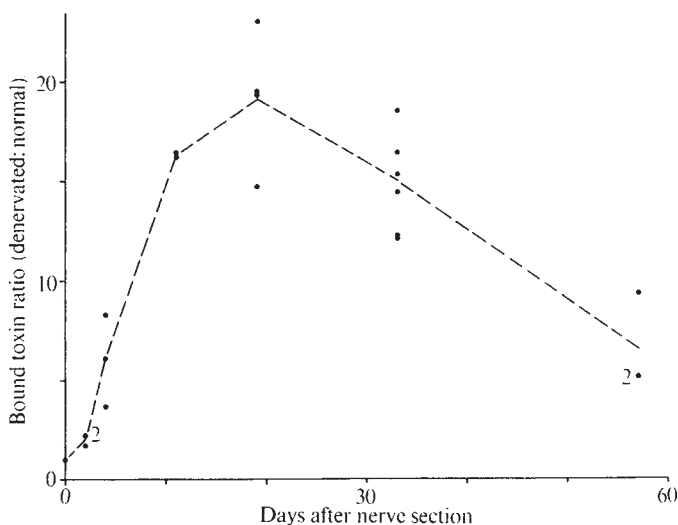


Fig. 3 Effect of denervation on number of binding sites. Left diaphragm muscles were denervated by section of the phrenic. At the indicated times after denervation, the muscles were exposed to labelled α -BuTX, 1 $\mu\text{g/ml.}$, for 4 h at 20° C, then washed and assayed for radioactive iodine. The normally innervated right hemidiaphragms and one sham operated left hemidiaphragm (point at 0 days) bound a mean of 3.95×10^{11} toxin molecules/hemidiaphragm. The ratio of toxin-binding sites in denervated to normal hemidiaphragms is shown on the ordinates.

Just as the ACh-sensitivity spreads to the entire muscle fibres, so does the binding of α -BuTX⁶. This was shown by incubating strips from a denervated diaphragm and from its opposite innervated control muscle in α -BuTX. After washing, the strips were fixed in glutaraldehyde, as previous experiments showed that all the bound toxin was retained after various

fixative procedures. Subsequently, the strips were divided into 2 mm segments and their labelled toxin content measured (Fig. 4). The increase in toxin bound to extra-junctional regions is large and, as there is no detectable ACh-sensitivity far from the end-plates in the normally innervated diaphragm², it seems likely that most of the small amount of toxin bound at these regions in normal muscles is unspecific. Nevertheless, after denervation the number of receptors in these regions increases by at least 200 times.

As extra-junctional regions develop a high ACh-sensitivity after denervation, and the ratio of total surface membrane to synaptic membrane areas is roughly 300, it is puzzling that the overall increase in toxin-receptive sites is only about twenty times. Perhaps the increase in receptors is not larger because, during the first weeks after denervation, the ACh-sensitivity of the extra-junctional region does not reach the level of sensitivity at the end-plate⁸, while subsequently the muscle becomes atrophic. Also, the juxta-synaptic region already has ACh-receptors² so after denervation the increase in receptors in this region will be comparatively smaller.

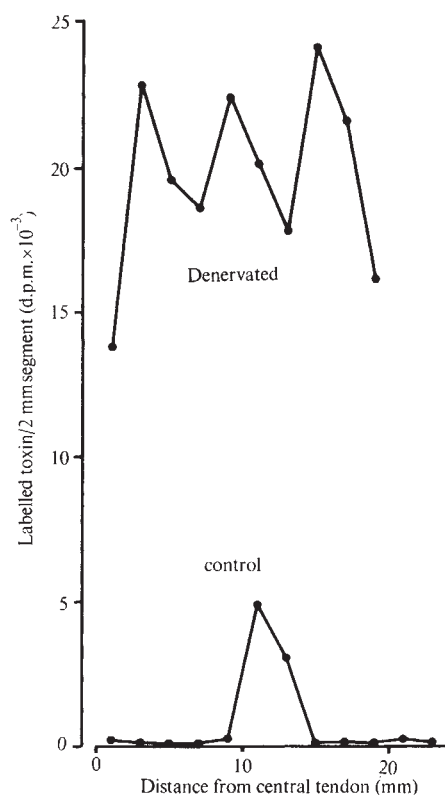


Fig. 4 Distribution of bound toxin along normal and denervated muscle fibres from the rat diaphragm. The end-points in the denervated strip have less toxin bound because of tapering of the strips.

After regeneration of neuromuscular synapses the ACh-sensitivity of extra-junctional regions again becomes very low¹⁷ and, as expected, the number of toxin-receptive sites is also decreased. For instance, two muscles were studied 71 days after crushing the phrenic nerve. With this procedure, the muscle fibres are denervated for approximately two weeks, and during that time they develop the generalized sensitivity to ACh, and are then rapidly reinnervated without allowing the muscle to become very atrophic. After exposure to toxin the reinnervated muscles had a mean of 5×10^{11} molecules of toxin per hemidiaphragm and a regenerated/normal ratio of 0.97, values similar to those observed in normal muscles.

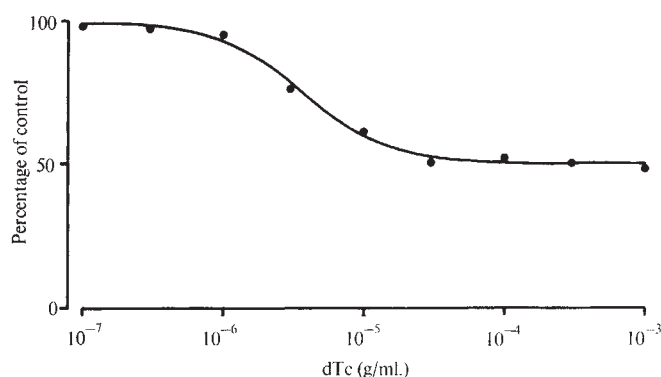


Fig. 5 Effect of *d*-tubocurarine (dTc) on binding of α -BuTX. One of each of nine paired frog sartorius muscles was equilibrated for 2 h in *d*-tubocurarine at the specified concentrations, and then exposed to α -BuTX while still in the dTc. All muscles were exposed to 1 μ g/ml. 125 I- α -BuTX for 1 h and then washed for 1 h in 10^{-4} g/ml. dTc without toxin to reduce further binding of toxin during the washing period. Ordinates represent the amount of toxin bound by the muscle incubated in dTc as a percentage of that of its paired muscle incubated without prior or simultaneous exposure to dTc.

Protection of Toxin-binding Sites by Curare

Chang⁴ and Chang and Lee⁵ have shown that *d*-tubocurarine (dTc) protects muscles against the postsynaptic action of the venom from *B. multicinctus*, and dTc would therefore be expected to reduce the binding of α -BuTX to muscles. To estimate the degree of this protection, frog sartorius muscles were equilibrated in various concentrations of dTc and were then exposed in addition to labelled α -BuTX (10^{-6} g/ml. for 1 h). The paired control muscle were separately exposed to the toxin without previous treatment with dTc. Fig. 5 shows that dTc decreased the binding of α -BuTX, and protected the ACh-receptors. With dTc concentrations higher than 10^{-5} g/ml. some muscle fibres still gave action potentials to nerve stimulation after both the toxin and dTc had been washed out.

An unexpected finding was that toxin-binding was not reduced below 50%, even with the highest dTc concentration used (Fig. 5). There seem to be, therefore, two classes of toxin binding sites, only one of which can be blocked by dTc at the concentrations studied, but the reason for this is not yet entirely clear. It could be that half the toxin binding sites are not ACh-receptors. This does not, however, seem very likely because the unspecific binding outside the end-plate is negligible, and because the binding of α -BuTX can be reduced further by ACh itself. A more plausible explanation is that there are two different membrane molecules, both capable of reacting with ACh and α -BuTX, but only one with a high affinity for dTc. While the pharmacological competition between ACh and dTc suggests that both act on the same site, it is also conceivable that there is a dTc-receptive molecule which does not have ACh-reactive sites, but which blocks the action of ACh through allosteric interactions. Differences in the distribution of the dTc-receptive sites could explain the lower dTc-sensitivity of extra-junctional receptors after denervation¹⁶ and also explain why the binding of radioactive curarine does not increase much after denervation¹³.

Desensitized Receptors

During prolonged exposure to a drug, the responsiveness of many tissues to the same or similar drugs is greatly reduced. In the case of skeletal muscles, Thesleff¹⁸ has shown that during continuous application of ACh, depolarization of muscle fibre membranes is not maintained (the resting potential gradually returns to its normal level, even though the ACh is still present). Thus muscle fibres become refractory to the action of ACh.

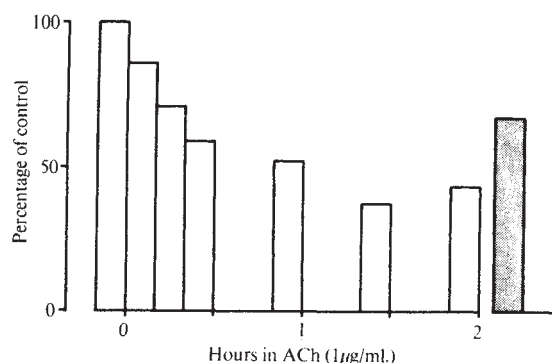


Fig. 6 Effect of desensitization of ACh-receptors on binding of α -BuTX. One of each pair of muscles was exposed to toxin (2 μ g/ml. for 10 min) after being in ACh (AChCl+neostigmine HBr both 1 μ g/ml.) for the indicated times. The other muscle from each pair was exposed to the toxin without prior treatment with ACh and served as control. All muscles were placed in ACh for a total of 3 h. One muscle (shaded bar) was removed from the ACh after 2 h and washed repeatedly for 5 min before being exposed to toxin, after which it was returned to ACh for 1 h.

This process of "desensitization" was later studied in more detail by Katz and Thesleff¹⁹ and others, and to explain it several hypotheses have been proposed¹⁹⁻²³.

In principle, the loss of action of ACh during sustained drug application could occur at any step from the ACh+receptor combination to the development of the ionic current responsible for membrane depolarization. An irreversible antagonist such as α -BuTX might produce some clues to this problem. For instance, if desensitization were to involve changes in the later steps, subsequent to the combination of ACh with the receptors, then the binding of α -BuTX in desensitized muscles might be expected to be the same as in a normal muscle. Alternatively, the change might occur at the receptor level and perhaps reduce the binding of toxin.

To examine these possibilities, muscles were exposed to ACh (10^{-6} to 10^{-4} g/ml.) in the presence of neostigmine methylsulphate (10^{-6} g/ml. to prevent hydrolysis of ACh by cholinesterases). Acetylcholine caused a marked and immediate depolarization of the end-plates, followed by a gradual recovery of the resting potential. One to three hours after commencing the application of ACh, the muscle bath was rinsed several times with normal Ringer to wash out the ACh, and approximately 10 min later the muscle was exposed to α -BuTX (10^{-5} – 3.6×10^{-5} g/ml.) for 5–10 min, and rinsed again several times. When these muscles had recovered from the desensitization caused by ACh, miniature end-plate potentials could be recorded from many surface fibres, and stimulation of the nerve evoked end-plate potentials which sometimes triggered muscle action potentials, in spite of having been exposed to the toxin. In contrast, muscles which had not been desensitized by ACh were blocked by the same treatment with α -BuTX. These experiments indicated clearly that desensitized muscles are less susceptible to the blocking action of α -BuTX, and further experiments were carried out to see if the binding of toxin was also reduced.

The binding of α -BuTX to muscles exposed to ACh for various times is progressively decreased as the muscles become desensitized (Fig. 6). During simultaneous exposure to ACh and α -BuTX, some competition for receptive sites may occur but this, however, would presumably have a faster time course than the decrease in binding illustrated in Fig. 6. A further indication that the decrease in binding is due to desensitization of receptors is that binding was still reduced 5 min after removing the ACh (shaded bar in Fig. 6). In other experiments it was found that a higher concentration of ACh (10^{-4} g/ml.) already reduced toxin binding maximally (81%) when applied simultaneously with the toxin, and that full recovery

of binding occurred with a time course roughly parallel to re-sensitization of the muscle fibre membrane.

In their model of desensitization, Katz and Thesleff¹⁹ proposed that receptors can exist in either an active or a refractory form. Their experimental results were best fitted to a scheme in which the affinity of the drugs for desensitized receptors was much higher than for resting receptors. More recently, Rang and Ritter²⁴ have found that certain bisquaternary drugs have a preferential affinity for desensitized receptors. It seems clear, however, that desensitization of the muscle fibre membrane by ACh reduces its ability to bind α -BuTX but it is not known how this is brought about. There are, of course, several possible explanations. For example, it could be that ACh molecules remain on the receptor for many seconds—instead of merely a fraction of a second—and that during that time receptors are unable to combine with the toxin. Another possibility is that activation of a receptor by ACh leads to a slowly-reversible change in the structure of the receptor, a change which prevents further combinations with ACh or toxin molecules.

Solubilization of the Toxin-binding Material

¹³¹I-labelled α -BuTX saturates binding sites in washed fragments of membranes from *Torpedo* electric tissue, and the toxin-tagged material can be "solubilized" (brought into a form which does not sediment during ultracentrifugation for several hours) with the non-ionic detergent, 'Triton X-100' (ref. 27).

Preliminary studies of the toxin binding sites of frog sartorius muscles by the same procedures (Fig. 7) indicate that the labelled substances in muscles and electric tissue are very

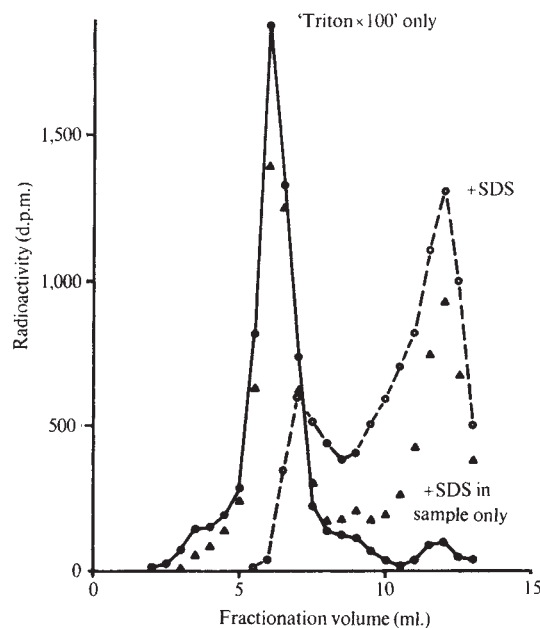


Fig. 7 Ultracentrifugation of toxin-binding material. Twelve frog sartorius muscles labelled with ¹³¹I- α -BuTX were homogenized in fifteen times their weight of 20 mM sodium phosphate buffer, pH 7.5. Large particles including cell membranes were sedimented at 10^5 g-min, and were extracted with 3 ml. of 1.5% 'Triton X-100' in 20 mM buffer for 2 h at 4°C. After recentrifugation, 0.5 ml. samples of the clear extract $\pm$ 2.5 mg sodium dodecyl sulphate (SDS) were centrifuged over linear density gradients (5–25% sucrose in 20 mM buffer + 1% 'Triton X-100' $\pm$ 0.5% SDS; 12.5 ml. in Spinco SW 40 Ti tubes) for 22 h at 39,000 r.p.m. Results are given as d.p.m. ¹³¹I per 0.5 ml. fraction; volumes are measured from the bottom of each centrifuge tube. The mobility of the toxin-binding material in 'Triton X-100', and its partially-reversible disaggregation in SDS, appear the same as that previously seen with toxin-binding material from *Torpedo* electric tissue²⁷.

similar. After toxin-labelled sartorius muscles were disintegrated with a high-speed blender in dilute buffer, 93% of the ^{131}I sedimented with cell particles during ultracentrifugation. Extraction of the sediment with 'Triton X-100' permitted quantitative recovery of the labelled substance in the supernatant fluid after recentrifugation. Examination of this material by density gradient centrifugation indicated that most of it sedimented in one peak, exactly as did similarly-treated toxin receptor from *Torpedo* electric tissue (compare Fig. 7 with Fig. 4B from ref. 27). In addition, SDS reversibly disaggregated the toxin-tagged material from muscles. These results indicate the feasibility of extensive purification and characterization of the muscle receptor for α -BuTX.

Toxin-receptive Sites and ACh-receptors

The correspondence between the pattern of ACh-sensitivity and the localization of bound toxin, in normal and denervated muscles, already indicates a strong similarity between ACh- and toxin-receptive sites; but confirmation that they are identical will have to await the elucidation of the detailed structure of the receptor. It should, however, be mentioned here that the effects of dTc, and in particular the experiments on muscles desensitized by ACh, suggest that ACh and α -BuTX act on the same site, or on sites close enough to allow interactions.

On the assumption that one toxin site is equivalent to one ACh-receptor, there are about 10^9 ACh-receptors in a frog muscle fibre. This is only a preliminary estimate, but already some interesting points emerge if considered in relation to the recent suggestion by Katz and Miledi^{25,26} that the depolarizing action of ACh is built up of elementary events with a calculated amplitude of 0.22 μV .

The neural transmitter is released in quanta, each of which would evoke about 2,000 elementary events; and a nerve impulse, releasing say 300 quanta, would evoke 6×10^5 elementary events. The question is still open whether one elementary event is caused by the activity of one or more receptors; but assuming, for the time being, that one active receptor is equivalent to one elementary event, a nerve impulse would activate less than one thousandth of the available receptors. Thus there seems to be a large number of spare receptors, even if each elementary event were to require the activation of ten receptors, which is not likely.

During external application of ACh, about 10^7 elementary events are required per second to maintain a depolarization of 10 mV (compare ref. 25). There would, therefore, be only sufficient receptors to maintain this depolarization for 100 s if each receptor were active only once. As such a depolarization can be maintained for longer, it follows that in this case some receptors are activated more than once. With larger doses of ACh a receptor might be active only once, or a few times, before the muscle is desensitized.

Another point of interest concerns the average density of receptors at an end-plate. Consider an average end-plate in the frog sartorius, with a total synaptic length of about 600 μm (unpublished results of R. M.). Assuming a 3 μm wide contact and 1,200 synaptic folds—each with 10 μm^2 of membrane—the total synaptic membrane would then be $1.4 \times 10^4 \mu\text{m}^2$. If 10^9 receptors occupied only the synaptic area, there would be in the order of 10^5 receptors per μm^2 . The average separation between receptor centres would then be about 3.2 nm, which would make it difficult to accommodate all the receptors in a monolayer and still leave space to accommodate the ionophores and the cholinesterase molecules known to be at the end-plate. The existence of extra-junctional receptors, however, must also be taken into account. Their distribution on the muscle fibre membrane can be estimated from the spatial pattern of ACh-sensitivity, but for the moment let us say that the synaptic area contains one-fifth of the receptors. In such a case the inter-receptor distance would still be only about 8.4 nm. But the ionophore need not be a separate molecular arrangement,

since the receptor macromolecule may provide the ionic "channel"; and the cholinesterase may not be located in the membrane matrix but instead in the basement membrane, as suggested by the recent experiments of Betz and Sakmann²⁸.

It is interesting that, as in eel¹⁴ and *Torpedo*²⁷ electric tissue, the calculated numbers of active sites of cholinesterase in frog²⁹, rat³⁰ and mouse³¹ muscle fibres seem to be within an order of magnitude of the number of toxin-binding sites. This correspondence is particularly interesting because the turnover number for the enzyme is very high (about 1–10 molecules per msec per active site²⁹) whereas the receptors desensitize and recover slowly.

Finally, the activity of an ACh-receptor can be predicted from preliminary estimates of the number of "effective" ACh-molecules. On collision theory, the number of collisions on a surface is $Z = nS \sqrt{\frac{kT}{2\pi m}}$, where n is the number of molecules per ml., S is the surface area and m the mass of the molecule³². During application of, say, 10^{-7} g/ml. of ACh to a sartorius muscle, and assuming that the ACh-receptor presents a target area of 20 $\text{\AA}^2$, and disregarding hydration, there would be 3.3×10^{13} collisions/s on the receptors (i.e., 3.3×10^4 collisions/s on each receptor). If the ACh depolarized the muscle by about 10 mV, requiring 10^7 elementary events/s, then only 3×10^{-7} of the collisions are effective. This may be so, not only because many collisions have a low energy, but also because in all likelihood there are restrictions on the orientation for effective collisions. If more than one active receptor is required to elicit an elementary event, or if the target area is smaller than assumed, the number of effective collisions would be greater than estimated; all of which tends to suggest that the ACh-receptor is a rather reactive molecule.

Received July 20, 1971.

- ¹ Katz, B., *The Release of Neural Transmitter Substances* (Liverpool University Press, 1969).
- ² Miledi, R., *J. Physiol.*, **151**, 24 (1960).
- ³ Brunton T. L., and Fayrer, J., *Proc. Roy. Soc., B*, **21**, 358 (1873); Brunton, T. L., and Fayrer, J., *Proc. Roy. Soc., B*, **22**, 68 (1874).
- ⁴ Chang, C. C., *J. Formosan Med. Assoc.*, **59**, 315 (1960); Chang, C. C., *J. Formosan Med. Assoc.*, **59**, 416 (1960).
- ⁵ Chang, C. C., and Lee, C. Y., *Arch. Intern. Pharmacodyn.*, **144**, 241 (1963).
- ⁶ Lee, C. Y., Tseng, L. F., and Chiu, T. H., *Nature*, **215**, 1177 (1967).
- ⁷ Pressman, D., and Eisen, H. N., *J. Immunol.*, **64**, 273 (1950).
- ⁸ Miledi, R., *Enzymes and Drug Action*, 220 (edit. by Monger, J. L., and de Reuck, A. W. S.) (Churchill, London, 1962).
- ⁹ Katz, B., and Miledi, R., *J. Physiol.*, **170**, 379 (1964).
- ¹⁰ Katz, B., and Kuffler, S. W., *J. Neurophysiol.*, **4**, 209 (1941).
- ¹¹ Miledi, R., *J. Physiol.*, **151**, 1 (1960).
- ¹² Krnjević, K., and Miledi, R., *J. Physiol.*, **140**, 472 (1958).
- ¹³ Waser, P., in *Molecular Properties of Drug Receptors*, 59 (edit. by Porter, R., and O'Connor, M.) (Churchill, London, 1970).
- ¹⁴ Changeux, J.-P., Kasai, M., and Lee, C. H., *Proc. US Nat. Acad. Sci.*, **67**, 1241 (1970).
- ¹⁵ Axelsson, J., and Thesleff, S., *J. Physiol.*, **147**, 178 (1959).
- ¹⁶ Beranek, R., and Vyskocil, F., *J. Physiol.*, **188**, 53 (1967).
- ¹⁷ Miledi, R., *J. Physiol.*, **154**, 190 (1960).
- ¹⁸ Thesleff, S., *Acta Physiol. Scand.*, **34**, 218 (1955).
- ¹⁹ Katz, B., and Thesleff, S., *J. Physiol.*, **138**, 63 (1957).
- ²⁰ Nastuk, W. L., Manthey, A. A., and Gissen, A. J., *Ann. NY Acad. Sci.*, **137**, 999 (1966).
- ²¹ Nastuk, W. L., and Parsons, R. L., *J. Gen. Physiol.*, **56**, 218 (1970).
- ²² Taylor, D. B., and Nedergaard, O. A., *Physiol. Rev.*, **45**, 523 (1965).
- ²³ Rang, H. P., and Ritter, J. M., *Mol. Pharmacol.*, **6**, 357 (1970).
- ²⁴ Rang, H. P., and Ritter, J. M., *Mol. Pharmacol.*, **6**, 383 (1970).
- ²⁵ Katz, B., and Miledi, R., *Nature*, **226**, 962 (1970).
- ²⁶ Katz, B., and Miledi, R., *Nature* (in the press).
- ²⁷ Miledi, R., Molinoff, P., and Potter, L. T., *Nature*, **229**, 554 (1971).
- ²⁸ Betz, W., and Sakmann, B., *Nature New Biology*, **232**, 94 (1971).
- ²⁹ Nachmansohn, D., *Chemical and Molecular Basis of Nerve Activity* (Academic Press, New York and London, 1959).
- ³⁰ Hall, S. W., and Kelly, R. B., *Nature*, **232**, 62 (1971).
- ³¹ Barnard, E. A., and Rogers, A. W., *Ann. NY Acad. Sci.*, **144**, 584 (1967).
- ³² Moelwyn-Hughes, E. A., *The Kinetics of Reactions in Solutions* (Clarendon Press, Oxford, 1947).

Molecular Evolution in the Descent of Man

MORRIS GOODMAN & JOHN BARNABAS

Department of Anatomy, Wayne State University School of Medicine, Detroit, Michigan 48207, and
Plymouth State Home and Training School, Northville, Michigan 48167

GENJI MATSUDA

Department of Biochemistry, Nagasaki University School of Medicine, Nagasaki

G. WILLIAM MOORE

Institute of Statistics, Biomathematics Program, North Carolina State University, Raleigh, North Carolina 27607

Three computer programs applied to data from phylogenetic trees and protein polymorphism can be used to find the rate of molecular evolution of different species. It can be shown that this is slower for higher primates than for other mammals.

It is likely that the level of molecular organization in higher animals is now more complicated than would have been possible in the first life forms of three billion years or so ago. Even after the emergence of the genetic code and the biochemical apparatus of translation, natural selection would have had to act over long stretches of evolutionary time to produce the sophisticated structures and functions of the multichained proteins now found in living organisms. To begin with, almost any point mutation in a gene coding for a polypeptide chain would have been tolerable; with the evolution of higher levels of molecular organization, however, increasingly stringent functional restraints would have markedly decreased the number of acceptable mutations.

The notion that the level of molecular organization places restraints on how fast genes evolve is implicit in Ingram's hypothesis¹ that the genes for alpha globin have changed more slowly than those for beta globins. The same alpha chain combines with different types of beta chains in the several tetrameric haemoglobins which are synthesized during the life of a mammal. Since there are more restraints on variations of the alpha chains than of the beta chains, fewer mutations would be tolerated at an alpha locus than at a beta locus and alpha genes would not evolve as rapidly as beta genes. In the same way, there may be differences in the rates of evolution of the globin genes at earlier and later evolutionary stages. The original single-chained globin would no doubt have been recognizable by its three-dimensional structure and haem binding site, but it would yet have had to acquire specific sites for subunit combination, for binding diphosphoglycerate, for allosteric modulation of structure to promote the Bohr effect and for combining with the plasma protein haptoglobin. With

these functions, natural selection tolerated fewer mutations in the globin genes. From this it follows that the primordial globin genes evolved more rapidly than the later globin genes of vertebrates, consonant with an earlier hypothesis that molecular evolution at a number of gene loci has decelerated, especially in the lineage leading to man²⁻⁴.

This hypothesis, followed by others^{5,6}, attributes divergent evolution in proteins to selectively neutral mutations but also implies that more mutations were neutral in primitive organisms than in descendant species. Thus biochemical adaptations in human antecedents must have increased with the development of new organs such as the placenta and the cerebral neocortex and the extra functional restraints would then decrease the chances for fresh mutations to accumulate. An immunological mechanism could have further restricted the degree of genetic variability in higher primate populations, for the haemochorial placenta (with its intimate apposition of foetal and maternal blood circulations) and the long gestation period would have increased the risk to the foetus from maternal immunizations to foetal allotypes.

We present evidence in this article that molecular evolution has indeed been slower in higher primates than in other mammals. In particular, from a detailed analysis of the phylogeny of globin genes, we support Ingram's hypothesis that the evolution of alpha globin genes has been slower than that of the beta genes. Available data on proteins also bear on the cladistic relationships of man and other mammals.

The most penetrating view of the molecular changes in individual genes during the descent of man is provided by comparing the amino-acid sequences of human proteins with homologous proteins in other organisms. We have followed the approach of Fitch and Margoliash⁷ in constructing gene phylogenetic trees from amino-acid sequence data and for this purpose have used three computer programs. The first program (MMUTD) calculates mutation distances for every pairwise comparison of aligned amino-acid sequences using a matrix based on the genetic code which gives for each amino-acid pair the minimum number of nucleotides that would need to be changed to bring about such a mutation. The second program⁶ (UWPGM) produces from a dissimilarity matrix of the mutation distances a dendrogram of the gene species in the set. This dendrogram is an initial approximation to a cladogram, that is a graph which depicts the order of ancestral branching in the gene species set. The UWPGM builds the

tree from the smallest to the largest branches in a series of pairwise clustering cycles, each time grouping together the two members of the set (either singleton species or joined species from a previous cycle) with the smallest mutation distance between them. If all the species in the set had evolved at a uniform, or nearly uniform, rate in their descent from a common ancestor, the dendrogram produced by the UWPGM would be the true cladogram⁹. But neither gene species nor animal species necessarily evolve at uniform rates. Thus, with the third computer program (DENDR) we construct alternative dendrograms. The DENDR program calculates the patristic mutation length* for each pair of adjacent nodes in a dendrogram, sums the patristic lengths through the sequence of nodes between species to calculate a reconstructed mutation distance for each pair of species in the set, and then compares the reconstructed mutation distances to the given mutation distances in the original dissimilarity matrix to calculate a coefficient for the entire dendrogram called "average percentage standard deviation" (APSD)⁷. Among the alternative dendrograms the one with least deviation between original and reconstructed mutation distances (that is, the lowest APSD coefficient) is considered the closest approximation of those tried to a true phylogenetic tree. This approach to finding the best fitting tree has proved capable of capturing the cladistic relationships among gene species even when markedly dissimilar amounts of mutational change characterize their descent from a common ancestor¹⁰.

Globin Phylogenetic Tree

Table 1 is the matrix of mutation values from all possible pairings of sixty-eight metazoan globin chain sequences¹¹⁻⁴⁷. These chains range in size from 135 to 153 amino-acid residues. They include, apart from carp alpha, chicken alpha, and mammalian alpha and non-alpha haemoglobin chains, a globin of lamprey, myoglobins of horse and sperm whale, and a globin of the insect chironomus. The chicken alpha chain is the first non-mammalian tetrapod haemoglobin chain to be sequenced¹⁷. It diverges less from human (and chimpanzee) alpha than from any other mammalian alpha (Table 1). This is not due to any special relationship between chicken and man, but, as will be shown, results from the marked conservatism of higher primate alpha chains.

The best tree so far obtained depicting the phylogeny of the globins is given in Fig. 1. Fig. 2 shows the best of forty-one alternatives tried for the restricted set of tetrapod alpha chains, and Fig. 3 the best tree of forty-five alternatives tried for the restricted set of mammalian non-alpha or beta-like globin chains. The APSD coefficient was lowered from 15.11 for the alpha tree constructed by the UWPGM to 11.14 for the tree in Fig. 2, and from 11.32 for the beta-like tree by the UWPGM to 8.40 for the tree in Fig. 3. The original tree for

the sixty-eight globin species constructed by the UWPGM had an APSD coefficient of 18.52. This was lowered by several points on trying a dendrogram which followed the branching order shown in Figs. 2 and 3, but which otherwise maintained the topology of the UWPGM tree. Shifting (a) the carp alpha branch from its UWPGM union with other alphas to a union with the line ancestral to both mammalian beta-like globins and tetrapod alphas, and (b) the lamprey globin branch from its UWPGM union with the branch ancestral to all the globin chains in vertebrate haemoglobins over to union with the myoglobin branch, lowered the APSD coefficient to 12.27 and produced the tree shown in Fig. 1. So far eighteen alternative configurations for the major branches of the large globin tree have been tried.

Course of Globin Evolution

The recognizable homologies between insect haem binding protein and vertebrate myoglobins and haemoglobins in codon sequence and in three-dimensional structure and functional properties⁴⁷ place the primordial globin gene deep in the Pre-Cambrian epoch before the protovertebrates had embarked on an independent course of evolution. Fig. 1 reveals that when man and lamprey still had a common ancestor, the primitive vertebrate genome already had more than one gene locus coding for globin chains; it was then that a gene duplication separated the ancestor of myoglobin genes from that of haemoglobin genes. Almost immediately after the myoglobin-haemoglobin duplication, the line leading to the globin in lamprey split off from the myoglobin side as a separate branch. Myoglobins are single chained proteins. Similarly, lamprey globins, including the one which has been sequenced, are single chained, although in certain physico-chemical conditions these lamprey globin chains form larger aggregates⁴⁵.

In the early bony fishes ancestral to the land vertebrates a haemoglobin gene duplicated to produce the split between the alpha and beta-like chain genes. This is depicted in Fig. 1 where the tetrapod alpha and beta-like lines separate from each other immediately after their separation from the carp alpha line. Much later in the lineage leading to therian mammals a gamma-beta chain gene duplicated to produce, on the one hand, the first separate ancestor of the genes for the kangaroo non-alpha chain, the human gamma chain (found in the haemoglobin of the human foetus) and the lemuroid non-alpha chains and, on the other hand, the separate ancestor for the mammalian beta chain genes. The first separate ancestor of the lemuroid (lemur and sifaka) non-alpha globin chains may have been a gamma-beta hybrid produced by unequal homologous crossing over between originally linked gamma and beta genes. There are two residues (13 Ser, 21 Glu) in kangaroo, lemur and sifaka chains and three additional residues in the lemur and sifaka chains (9 Ala, 50 Ser, 52 Ser) which are homologous to human gamma chain. Furthermore, lemur and sifaka chains resemble more human gamma from the N-terminal end than human beta and resemble more human beta from the C-terminal end than human gamma.

At the nodes of Fig. 2 for the two most ancient branching points among mammalian alpha genes, ancestral rabbit and tree shrew genes diverged from each other immediately after their separation from the branch to all other mammalian alphas. Tree shrew alpha actually shows a greater mutation distance from rabbit, and rabbit from tree shrew, than either shows from other mammalian alpha chains (Table 1). This type of pattern, which resembles that of the human gamma, kangaroo and lemuroid non-alpha branch of the beta-like tree, suggests that alpha gene duplications had occurred in the early therian mammals producing ancestral rabbit and tree shrew genes and also the ancestor of other mammalian alphas. Recent duplications of alpha genes are known to occur in present day mammals, for example, in buffalo⁵⁰, horse²⁷, goat⁵², macaques⁵⁴, mouse⁵⁶ and probably rabbit¹⁸.

During eutherian phylogeny, beta chain genes duplicated

* The patristic mutation lengths associated with any node, N , on a dendrogram are calculated as follows. Any node N divides the species of which it is the common ancestor into two sets: set A and set B . All remaining species under study comprise set C . Let D_{ab} be the average of dissimilarity values for pairs of species such that the first species is a member of set A and the second species is a member of set B , and let D_{ac} and D_{bc} be similarly calculated. The length from set A to node N is called a ; the length from set B to node N is called b ; and the length from set C to node N is called c . These lengths are calculated by the following formulas:

$$a = \frac{D_{ab} + D_{ac} - D_{bc}}{2}$$

$$b = \frac{D_{ab} + D_{bc} - D_{ac}}{2}$$

$$c = \frac{D_{ac} + D_{bc} - D_{ab}}{2}$$

For the special case that N is the apex of the tree (that is, the common ancestor of all species under study): $a = b = \frac{D_{ab}}{2}$

c is undefined.

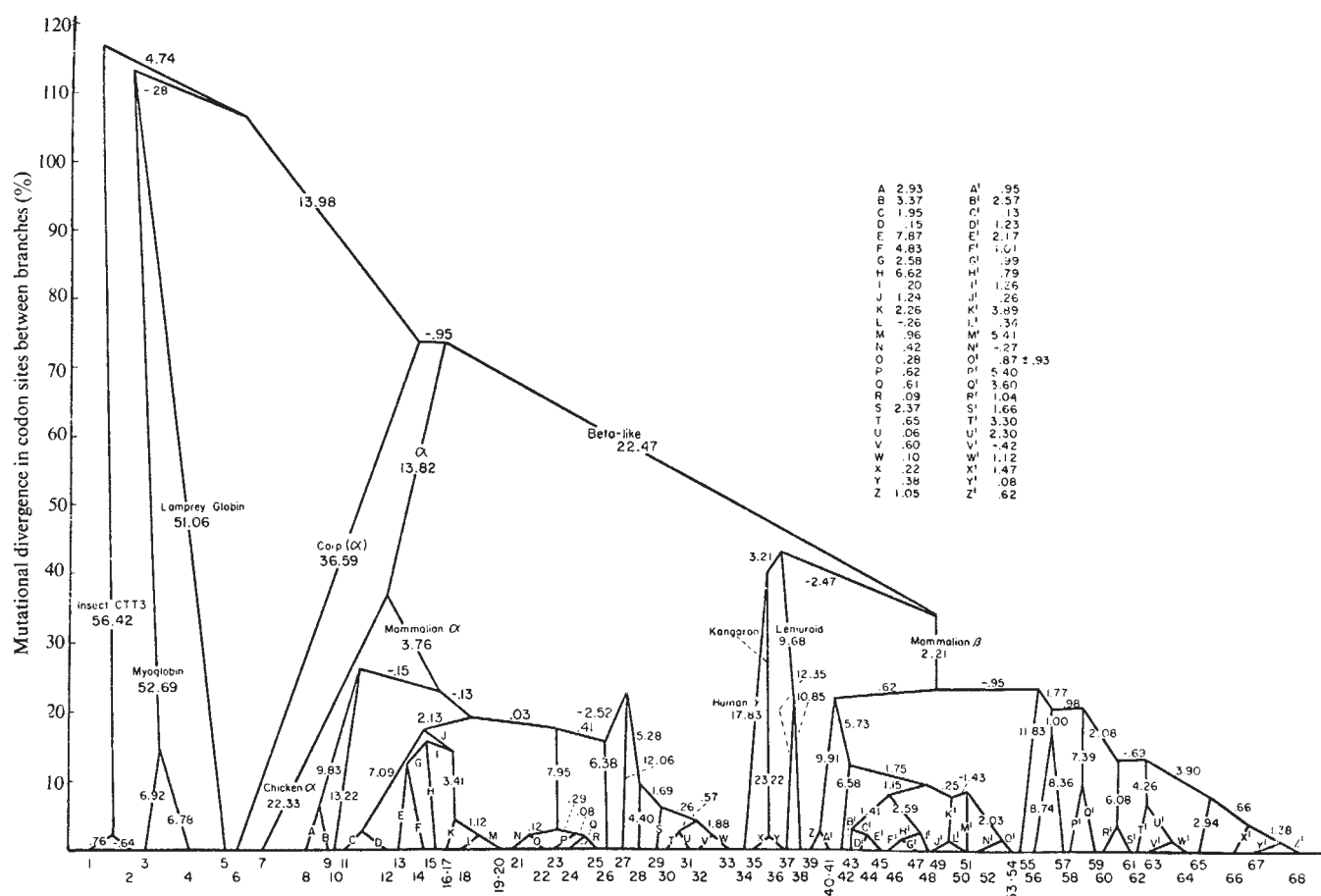


Fig. 1 Globin phylogenetic tree. 1, Insect CTT-3 (*Chironomus thummi*)¹¹; 2, insect CTT-3 (sub)¹¹—alternative amino-acids (39 Thr, 57 Ile) were used for ambiguous positions; 3, myoglobin sperm whale¹³; 4, myoglobin horse—sequence alignment as given in ref. 14; 5, globin lamprey¹⁵; 6, alpha chicken¹⁶; 7, alpha chicken¹⁷; 8, alpha rabbit¹⁸; 9, alpha rabbit (sub)¹⁸—alternative amino-acids (29 Leu, 48 Leu, 70 Thr, 76 Val, 80 Ser, 113 His) were used for ambiguous positions; 10, alpha tree shrew¹⁹; 11, alpha mouse NB^{20,21}; 12, alpha mouse C-57Bl^{20,21}; 13, alpha sifaka (*Propithecus verreauxi*)¹⁹; 14, alpha lemur (*Lemur fulvus*)¹⁹; 15, alpha bush baby (*Galago crassicaudatus*)¹⁹; 16, alpha *Macaca mulatta*²²; 17, alpha *Macaca fuscata* (G. Matsuda, unpublished); 18, alpha gorilla²³; 19, alpha chimpanzee²⁴; 20, alpha human^{25,26}; 21, alpha donkey²⁷; 22, alpha horse slow (24 Phe)²⁷; 23, alpha horse fast (24 Phe)²⁷; 24, alpha horse fast (24 Tyr)²⁷; 25, alpha horse slow (24 Tyr)²⁸; 26, alpha pig—sequence alignment as given in ref. 14; 27, alpha llama—sequence alignment as given in ref. 14; 28, alpha bovine²⁹; 29, alpha goat II (non-allelic)³⁰; 30, alpha goat B (allelic)³⁰; 31, alpha goat A³⁰; 32, alpha sheep D³¹; 33, alpha sheep A^{32,33}; 34, gamma human³⁴; 35, beta kangaroo³⁵; 36, beta kangaroo-2 (2 Gln)³⁵; 37, beta sifaka¹⁹; 38, beta lemur¹⁹; 39, beta mouse AKR³⁶; 40, beta mouse Sec³⁶; 41, beta mouse C-57Bl³⁶; 42, beta rabbit³⁷; 43, beta squirrel monkey (*Saimiri sciureus*)³⁸; 44, beta tamarin (*Saguinus nigricollis*)³⁸; 45, beta spider monkey (*Ateles geoffroyi*)³⁸; 46, delta squirrel monkey³⁸; 47, delta tamarin³⁸; 48, delta spider monkey³⁸; 49, beta *Macaca fuscata*³⁹; 50, beta *Macaca mulatta*²²; 51, delta human⁴⁰; 52, beta gorilla²³; 53, beta chimpanzee²⁴; 54, beta human²⁵; 55, beta horse slow⁴²; 56, beta pig—sequence alignment as given in ref. 14; 57, beta llama—sequence alignment as given in ref. 14; 58, beta bovine foetal⁴²; 59, beta sheep foetal⁴³; 60, beta bovine A⁴⁴; 61, beta bovine B⁴⁴; 62, beta sheep barbary C⁴⁵; 63, beta goat C⁴⁷; 64, beta sheep C⁴⁶; 65, beta sheep B⁴⁶; 66, beta sheep A⁴⁶; 67, beta goat A⁴⁷; 68, beta goat A' (allelic)⁴⁷.

independently in separate mammalian lines and certain of the descending duplicated genes ultimately coded for new proteins. For example, postnatal human blood contains in addition to its major haemoglobin (A) a minor haemoglobin (A₂) in which a different beta chain, called delta chain^{1,57}, combines with alpha chain. Similarly, the ceboid monkeys have a minor haemoglobin^{38,58} with a beta chain (again called delta) different from the beta chain of the principal ceboid haemoglobin. Fig. 3 depicts in the early Anthropeidea, shortly after the branching apart of ceboids and catarrhine primates, two independent beta gene duplications: one on the catarrhine side coincided with the hominoid-cercopithecoid split to give rise to hominoid delta, and the other on the ceboid side produced the ancestor of ceboid deltas. Independent beta gene duplications also occurred during the evolution of the ruminants. Before the splitting apart of bovines and caprines, a descendant gene from a beta duplication in the early ruminant line began to code for the bovid foetal beta chain. The caprines (but not the bovines) have, like hominoids and ceboids, a second adult haemoglobin^{45–47,59} (in this case,

called haemoglobin C) normally present in negligible quantities, but synthesized in large amounts in animals under severe anaemic stress. In this second adult caprine haemoglobin, the beta C chain is found rather than the regular beta chain of caprine haemoglobin A. The ancestral A-C gene duplicated in the caprine line to give rise to the linked A and C gene loci shortly after the ancestral separation of bovines and caprines (Fig. 3).

Later in caprine evolution, at some point before the ancestral separation of sheep and goat, a mutation at the A locus produced the first separate ancestor of the present day sheep beta B gene. The period of separate evolution of sheep betas B and A seems to be greater than that of sheep and goat beta As. Present day sheep beta B differs from sheep or goat beta A by seven point mutations, whereas sheep beta A differs from goat beta A by four point mutations (Table 1). The sheep A and B-beta results illustrate that typical allelic proteins within a mammalian population can differ by several or more amino-acids. Bovine A and B beta alleles provide another example of such allelic variation; they differ by four point mutations

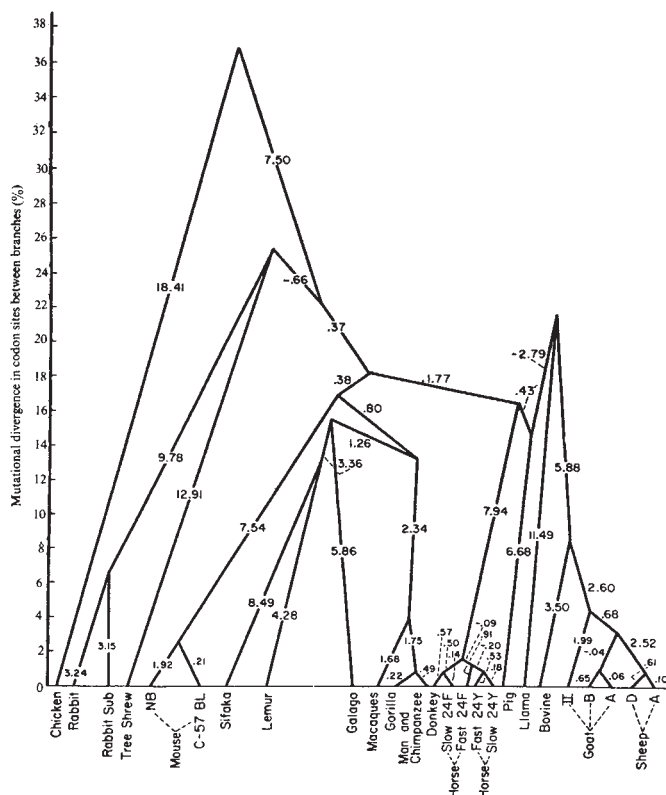


Fig. 2 Chicken and mammalian alpha globin phylogenetic tree.

(Table 1). Allelic human haemoglobins, on the other hand, differ from common human haemoglobin A mostly by only single point mutations.

The difference between bovid and human populations with respect to the kind of allelic variation expressed may be because the human foetus is at greater risk from maternal isoimmunizations than the bovid foetus. Bovids, like many mammals, have placentas of the epitheliochorial type⁶⁰ which separate the maternal and foetal vascular systems by several layers of tissue and thereby minimize the chance of maternal immune attacks on the foetus. In contrast the haemochorial placenta of the higher primates maximizes the opportunity for such attacks. Thus, the type of allelic beta chains with multiple differences in bovids, if found in man, could give rise to maternal immune reactions and be selected against. The first placentas to evolve in mammals were probably of the epitheliochorial type⁶⁰, so that the early mammals would not have been subjected to the immunological mechanism restricting genetic variability which we postulate for higher primate populations.

Rates of Globin Evolution

The patristic distance data, tabulated in Table 2, demonstrate that rates of evolution have varied markedly among vertebrate globin genes. The mammalian alpha genes diverge the least from the common vertebrate globin ancestor and the myoglobin genes; the group of non-alpha kangaroo, lemuroid, and human gamma genes diverge the most; lamprey globin, carp alpha, and chicken alpha each show more mutational change from this common ancestor than does any mammalian alpha (Fig. 1; Table 2, column 4).

While the patristic mutation lengths from Fig. 1 reveal that the proportion of codon positions showing mutations varied markedly among the principal branches of vertebrate globins, they do not show any especially marked variation among mammalian alpha genes, or among typical mammalian beta genes. But the patristic mutation lengths for the mammalian alpha portion of the globin tree from Fig. 2 and for the

mammalian beta portion from Fig. 3 reveal that rates of evolution have indeed varied markedly both among mammalian alpha genes and among mammalian beta genes (Table 2, column 3). In both sets of mammalian genes, the most slowly evolving lines were those which led to man and other higher primates. In the large globin series (Fig. 1) the mutation distance between very anciently separated species, such as between any alpha and any beta globin chain, would be grossly underestimated due to uncounted multiple mutations at the same nucleotide sites. Thus, differences in rates of evolution among homologous mammalian genes, which are reflected in the patristic mutation lengths shown in Figs. 2 and 3, are smoothed out in the lengths shown in Fig. 1 where the apportioning of the mutation distances between the more recently separated genes is disproportionately affected by the mutation distance comparisons to the many, very anciently separated globin genes. Kimura⁶¹ concluded that homologous genes evolved at uniform rates because about the same number of amino-acid substitutions separate any mammalian beta from any mammalian alpha and from carp alpha. But he failed to consider that the further in the past the separation of two lineages the larger the number of multiple mutations that must have occurred at the same nucleotide sites.

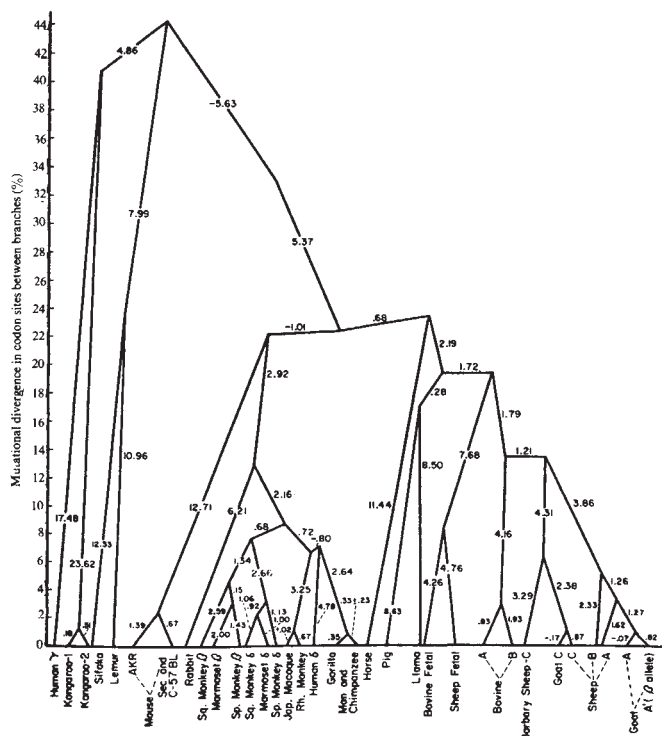


Fig. 3 Mammalian beta-like globin phylogenetic tree.

To detect a larger proportion of the accumulated mutations in a gene lineage, we constructed ancestral sequences (Table 3) for branching points of the globin phylogenetic tree and, using the MMUTD program, determined the mutation distances through the consecutive ancestors to the contemporary polypeptide chains. The mutational change in codon sites from the node for ancestral carp-tetrapod haemoglobin chains to the node for ancestral chicken-mammal alphas and from the latter to the ancestral node for mammalian alpha chains was 23.9 and 9.2% respectively. In the descent of the beta-like line, the mutational change from the ancestral carp-tetrapod node to the ancestral gamma-beta node and from this node to the ancestral node for typical mammalian beta chains was 53.2 and 2.0% respectively. The further mutational change in descent of each eutherian alpha and beta line is given in column 2 of Table 2. These results show

Table 2 Percentage Mutational Divergence of Vertebrate Globins during Descent

Globin species	From ancestral eutherian mammal MMUTD†	From ancestral eutherian mammal PML	From ancestral Hb-Mb PML
Human alpha	6.3	6.13	39.34
Chimp alpha *	6.3	6.13	39.34
Gorilla alpha *	7.0	5.86	38.12
Mulatta alpha	7.1	5.57	41.68
Fuscata alpha	7.1	5.57	41.68
Mouse C57 alpha	8.5	8.50	39.75
Mouse NB alpha	10.6	10.21	41.65
Goat A alpha	10.6	8.96	36.26
Lemur alpha *	11.3	10.45	41.46
Bush baby alpha *	11.3	8.67	40.67
Goat B alpha *	11.3	9.55	36.85
Goat II alpha	11.3	13.04	37.74
Bovine alpha *	11.3	9.16	38.08
Horse slow alpha	12.0	9.97	40.08
Horse fast alpha *	12.7	10.32	39.44
Sheep A alpha	12.7	11.56	37.61
Horse slow 24F alpha *	12.7	10.72	38.86
Horse fast 24F alpha *	13.4	10.99	39.37
Donkey alpha *	13.4	11.15	39.00
Sheep D alpha *	13.4	12.07	41.71
Pig alpha *	13.4	9.25	37.30
Rabbit alpha *	15.6	12.36	43.22
Rabbit (sub) alpha *	16.3	12.27	43.66
Sifaka alpha *	17.7	14.66	44.50
Tree shrew alpha *	17.7	12.25	43.68
Llama alpha *	19.1	11.27	40.46
Chicken alpha	—	—	49.18
Human beta	7.5	7.19	48.46
Chimp beta *	7.5	6.73	46.60
Squirrel monkey beta	7.5	8.68	50.94
Gorilla beta *	8.2	6.98	46.39
Fuscata beta *	8.2	8.06	50.21
Spider monkey beta	8.2	7.67	50.67
Tamarin beta	8.8	8.24	49.73
Mulatta beta	8.9	8.71	50.29
Rabbit beta	8.9	8.12	50.64
Human delta *	9.5	8.77	50.04
Tamarin delta	10.2	9.33	51.33
Spider monkey delta	10.2	8.54	50.81
Squirrel monkey delta	10.9	9.39	51.35
Pig beta *	14.2	11.78	48.27
Bovine A beta	16.4	12.47	48.71
Llama beta *	17.7	11.65	47.89
Bovine B beta	17.8	11.37	49.33
Sheep B beta	18.4	13.78	47.76
Horse beta	19.0	12.02	48.59
Goat A beta *	19.1	12.64	46.92
Sheep A beta	19.8	14.33	46.93
Bovine foetal beta	19.8	16.53	52.30
Goat C beta *	20.3	14.11	47.46
Mouse AKR beta *	20.4	13.09	49.29
Mouse Sec and C-57 beta *	20.7	12.37	49.19
Goat A' beta *	21.2	13.23	47.46
Sheep C beta	21.7	15.15	48.58
Sheep Barb. C beta *	21.7	15.19	48.46
Sheep foetal beta *	24.2	17.03	50.50
Kangaroo-1 beta	—	—	59.68
Kangaroo-2 beta	—	—	59.84
Human gamma	—	—	54.07
Lemur beta *	—	—	53.56
Sifaka beta *	—	—	55.06
Carp alpha	—	—	50.57
Lamprey globin	—	—	50.78
Mb-horse	—	—	59.47
Mb-sperm whale	—	—	59.33
Insect CTT-3	—	—	—
Insect CTT-3 (sub)	—	—	—

* Sequence in part or wholly by composition and homology.

† Values of rabbit alpha, rabbit (sub) alpha, tree shrew alpha, mouse NB alpha and mouse C-57 alpha are directly from ancestral mammalian alpha. Values of prosimian alphas are through ancestral primate alpha node while those of catarrhine alphas are through both ancestral primate alpha node and ancestral catarrhine alpha node. Values of mouse AKR beta, mouse Sec and C-57 betas and rabbit beta are directly from ancestral mammalian beta while those of ungulate betas and primate betas are through ancestral ungulate beta node and ancestral Anthroidea beta node, respectively.

that after the alpha-beta globin gene duplication in early vertebrate history, after the ancestral separation 380 million years ago of teleosts and tetrapods, many more mutations accumulated during the descent towards mammals in the beta-like line than in the alpha line. Moreover, during eutherian phylogeny, both alpha and beta chain evolution was much slower (on the average, almost by half) in higher primates than in other mammals. Furthermore, from the ancestral carp-tetrapod node about as much mutational change accumulated up to the ancestral chicken-mammalian alpha branching point as after this point in the descending mammalian alpha line. Yet one has to go back about 280 million years ago to the early reptiles to find the most recent common ancestor of birds and mammals. Thus the evidence suggests that the descending alpha genes evolved at a more rapid rate in earlier less advanced vertebrates than in later higher vertebrates.

The patristic distance data on beta genes show that ceboid delta, bovid foetal beta, and caprine C beta initially evolved more rapidly than the genes from which they were duplicated (Fig. 3), but later in descent their rates of evolution slowed. Note (Table 1) that ceboid deltas actually diverge less from each other than ceboid betas, that sheep and bovine foetal betas diverge less from each other than sheep and bovine adult betas, and that sheep and goat C betas diverge less than sheep and goat A betas. Presumably mutations had accumulated freely at the duplicated loci until certain fortuitous mutations resulted in the emergence of new proteins with useful functions. Then natural selection not only caused these particular advantageous mutant genes to spread rapidly through the populations in which they occurred but also drastically slowed their further evolution.

Following the gamma-beta gene duplication, more mutational change occurred in the descent of human gamma than in most typical eutherian beta chains. Nevertheless amino-acid composition data⁶² indicate that slightly less divergence exists between gamma chains of man and baboon than between either beta or alpha chains of these animals. Thus when sequence data become available on gamma chains of different higher primates, the pattern of decelerating evolution will probably be found again. This pattern is presented by the myoglobin genes, for the divergence between horse and sperm whale myoglobins (Fig. 1 and Table 1) is no more than that between any two alpha chains belonging to different eutherian orders; yet the overall mutational change in the descent of the myoglobin branch is larger than in the branch containing the various chains of vertebrate haemoglobin (Table 2, column 4). Ohno⁶³ describes early vertebrate history as a time when tetraploidization and bursts of gene duplication were occurring in our ancestors; at the later, mammalian stage specialized functions were found for pre-existing redundant genes, but the level of genome organization was such that viable tetraploidization was no longer possible. If mutations accumulate more readily in silent genes than in functional ones, this is equivalent to the hypothesis that molecular evolution decelerated during the descent of man.

In lemurs and other prosimians, foetal haemoglobin seems to be the same as adult haemoglobin⁶⁴. But in the lineage leading to higher primates a specialized function, coding for the non-alpha chain of foetal haemoglobin, was found for the gamma locus. A further advance took place when from certain beta duplications of the early Anthroidea functioning delta genes and a new haemoglobin type emerged in later Ceboidea and Hominoidea. The new level of organization imposed additional functional restraints, which increased the likelihood that new mutations would be detrimental, thereby slowing the further evolution of the haemoglobin genes. While the alpha and beta chains of man are among the most slowly evolved globins, those of the kangaroo and lemuroids are among the most rapidly evolved. As previously discussed, an additional selective factor, maternal isoimmunizations to foetal antigens, might have also acted to decelerate molecular

Table 3 Probable Ancestral Residues for Vertebrate Globin Chains at Different Nodes during Descent

Residue position	11	13	14	15	16	18	19	21	22	23	24	25	27	28	29	30	31	32	34	36	38	40	42	43	44	45	46	48	50	51	52	54
β -Mammal	His	Thr	Ala	Glu	Glu	Ala	Ala	Thr	Ala	Leu	Trp	Gly	Val	Asx	—	—	Val	Asp	Val	Gly	Ala	Gly	Leu	Leu	Val	Val	Tyr	Trp	Gln	Arg	Arg	Glu
γ - β -Mammal	His	Thr	Ala	Glu	Glu	Ala	Ala	Thr	Ala	Leu	Trp	Gly	Val	Asn	—	—	Val	Asp	Val	Gly	Ala	Gly	Leu	Leu	Val	Val	Tyr	Trp	Gln	Arg	Arg	Glu
α -Mammal	—	Ser	Pro	Ala	Ala	Thr	Ala	Lys	Ala	Ala	Ala	Gly	Ile	Gly	Gly	His	Ala	Gly	Tyr	Ala	Ala	Glu	Met	Phe	Leu	Ser	Phe	Thr	Lys	Thr	Pro	
α -Chicken	—	Ser	Ala	Ala	Ala	Ala	Ala	Lys	Ala	Ala	Ala	Ala	Ile	Gly	Gly	His	Ala	Glu	Tyr	Ala	Ala	Glu	Met	Phe	Ile	Gly	Phe	Thr	Lys	Thr	Pro	
α - β -Carp	—	Ser	Ala	Ala	Ala	Ala	Ala	Lys	Ala	Ala	Ala	Ala	Ile	Gly	Gly	His	Ala	Asp	Tyr	Gly	Ala	Gly	Met	Phe	Ile	Gly	Tyr	Thr	Lys	Thr	Ala	
β -Mammal	55	57	61	62	63	64	65	66	68	69	70	71	73	79	81	83	84	85	86	88	90	92	93	94	97	98	99	100	103	106	107	110
γ - β -Mammal	Ser	Gly	Ser	Ala	Asx	Ala	Val	Met	Asn	Pro	Lys	Val	Ala	Leu	Asx	Phe	Ser	Asp	Gly	Asx	Leu	Asn	Leu	Lys	Thr	Phe	Ala	Gln	Glu	Cys	Asp	His
α -Mammal	Ser	Gly	Ser	Ala	Ser	Ala	Val	Met	Asn	Pro	Lys	Val	Ala	Leu	Asx	Phe	Ser	Asp	Gly	Asx	Leu	Asn	Leu	Lys	Thr	Phe	Ala	Gln	Glu	Cys	Asp	His
α -Chicken	His	—	His	—	—	—	—	—	Ser	Ala	Gln	Val	Gly	Ala	Asp	Leu	Thr	Asn	Ala	Gly	Leu	Asp	Leu	Pro	Ala	Leu	Ser	Ala	Asp	Ala	His	Arg
Mammal	His	—	His	—	—	—	—	—	Ser	Ala	Gln	Val	Gly	Ala	Asp	Leu	Thr	Asn	Ala	Gly	Leu	Asp	Leu	Pro	Ala	Leu	Ser	Ala	Asp	Ala	His	Arg
α - β -Carp	His	Gly	Pro	—	—	—	—	—	Ser	Ala	Gln	Val	Gly	Ile	Asp	Val	Ser	Asp	Ala	Gly	Leu	Asp	Leu	Glu	Ala	Leu	Ser	Ala	Glu	Ala	His	Arg
β -Mammal	114	117	120	121	122	123	124	125	126	128	129	131	132	133	134	135	136	138	140	142	143	144	147	148	149	151	152	153	155	156	159	
γ - β -Mammal	Glu	Arg	Gly	Asn	Val	Leu	Val	Ile	Val	Ala	His	Phe	Gly	Lys	Glu	Phe	Thr	Glx	Gln	Ala	Tyr	Gln	Val	Ala	Gly	Ala	Asn	Ala	Ala	His	His	
α -Mammal	Glu	Arg	Gly	Asn	Val	Leu	Val	Ile	Val	Ala	His	Phe	Gly	Lys	Glu	Phe	Thr	Glx	Gln	Ala	Phe	Gln	Val	Ala	Gly	Ala	Asn	Ala	Ala	His	His	
α -Chicken	Val	Lys	Ser	His	Cys	Leu	Leu	Val	Thr	Ala	Ala	Leu	Pro	Ala	Asp	Phe	Thr	Ala	His	Ser	Leu	Asp	Leu	Ala	Ser	Ser	Thr	Val	Thr	Ser	Arg	
Mammal	Val	Lys	Ser	His	Cys	Leu	Leu	Val	Thr	Ala	Ser	Leu	Pro	Ala	Glu	Phe	Thr	Glu	His	Ser	Leu	Asp	Leu	Ala	Ala	Ser	Thr	Val	Thr	Ser	Arg	
α - β -Carp	Glu	Lys	Ser	Asn	Ala	Leu	Val	Val	Val	Ala	Ser	Leu	Pro	Ala	Glu	Phe	Thr	Glu	His	Ser	Val	Asp	Leu	Ala	Ala	Ala	Asn	Ala	Thr	Ser	Arg	

Amino-acid residues of vertebrate globins are aligned so as to maximize homology by introducing the necessary gaps (refs. 14 and 15). The residue positions are numbered from 1 to 165 in order to accommodate lamprey globin. In this alignment carpo alpha, tetrapod alphas, and mammalian betas begin at position 159, with a gap at position 96. Carpo alpha and tetrapod alphas have in addition gaps at position 11 and at position 62 through 66. Further gaps introduced are: tetrapod alphas at positions 57 and 78; mammalian betas at positions 29, 30, and 78; carp alpha at position 73. Residue positions omitted are the same as chicken¹⁷ when aligned as described. Probable ancestral residues of mammalian alpha and beta chains at branching points within the span of eutherian phylogeny are given in the previous investigation¹⁶. Ancestral sequences were reconstructed by approximation after the rules described by Fitch and Margolish⁷. In brief, an ancestral residue is selected if it gives fewer mutations in the lines of descent than any other residue which could be selected and, where more than one residue meets this criterion, distributes the mutations as equally as possible between the lines of descent.

evolution in the higher primates. The opportunity for such attacks would be virtually absent in kangaroo, and minimal in lemuroid primates which have epitheliochorial type placentas⁶⁵ rather than the haemochorial type of higher primates.

Cladistic Relationships of Man and Other Mammals

The phylogenetic trees constructed from amino-acid sequence data not only reveal differences in relative rates of evolution among homologous genes but also provide evidence on the cladistic relationships of the animal species in which the genes occur. The branching topologies of alpha chain genes and beta chain genes depict a very close relationship, with no detectable mutational divergence, between man and chimpanzee, and between this man-chimpanzee branch and gorilla, in which the divergence is only one point mutation per chain. A beta phylogenetic tree, which includes the partially sequenced gibbon beta chain, joins gibbon to the branch leading to chimpanzee, man, and gorilla, and then joins cercopithecoïd (macaques) and hominoid branches¹⁰. Orang-utan haemoglobin chains have not been sequenced. Amino-acid composition data, however, show orang-utan diverging from the branch of African apes and man⁶⁶. In the beta phylogenetic tree (Fig. 3), the ceboids branch away from the line leading to cercopithecoïds and hominoids just before these two catarrhine groups branch apart. The phylogenetic tree of alpha genes (Fig. 2) joins the prosimians (galago together with the lemuroids, lemur and sifaka) to the line leading to higher primates, next joins the mouse branch to the base of the primate branch, and then joins the ancestral mouse-primate lineage with that of the ungulates. Both alpha and beta trees join caprines to bovines, llama and pig lines to the bovids, the horse branch with these artiodactyls, and then these ungulates with the lineage descending towards primates. Lemuroids do not produce a globin chain directly homologous to mammalian beta chains, so the phylogenetic tree of beta genes cannot provide cladistic evidence on the lemuroids. Otherwise the topology of the beta tree is similar to the alpha, except that rabbit beta joins primate betas before mouse.

Sequence data have also been obtained on mammalian fibrinopeptides A and B. They contain no more than forty amino-acid residues, all of which, with the exception of one or two invariant sites, have accepted amino-acid substitutions without harm to the function of fibrinogen. Thus these peptide sequences have been able to undergo such extensive divergent evolution that they are capable of depicting cladistic relationships among present day mammals. A matrix of mutation values for thirty-six mammalian combined A and B fibrinopeptide sequences⁶⁷⁻⁷⁵ is given in Table 4, and the best tree of thirty alternatives tried portraying the phylogeny of these sequences is shown in Fig. 4. The APSD coefficient of this tree is 10.80 compared with 14.63 for the UWPGM tree.

The branching arrangement among ungulate fibrinopeptides is essentially the same as that for ungulates in the alpha and beta globin phylogenetic trees, except that more ungulate species are

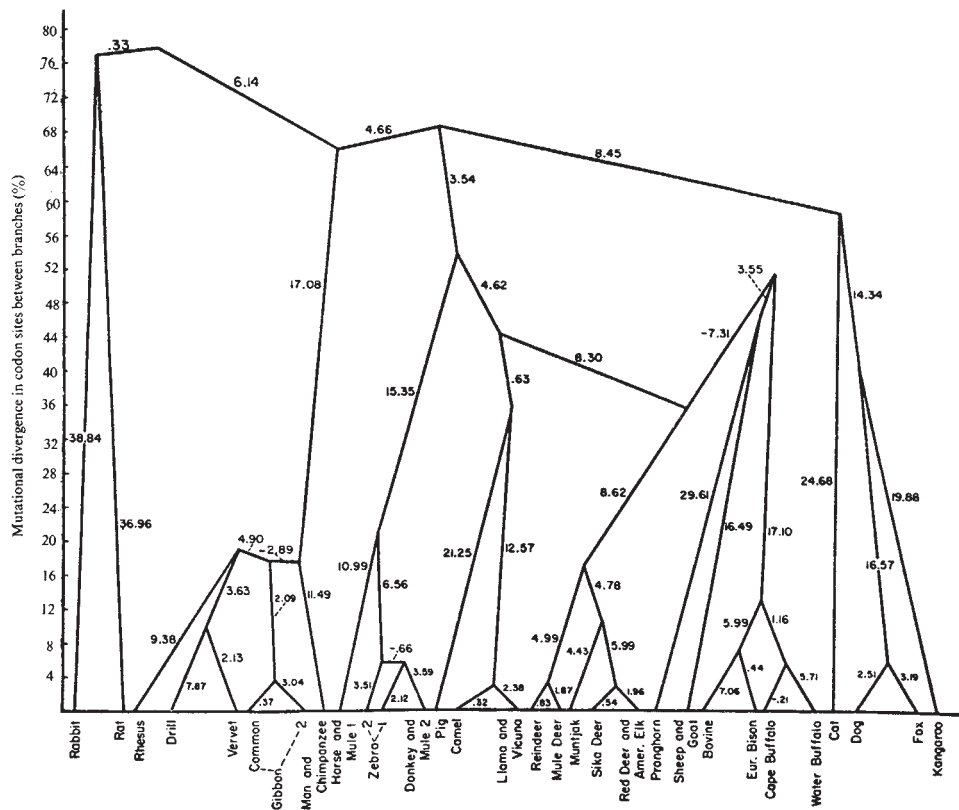
represented. Pronghorn and caprines join bovines and then these bovids join cervids (reindeer and various deers). Yet caprines and cervids on the average show less mutation distance from each other than from bovines (Table 4). Doolittle and Blombäck⁷⁶ discussed the possibility that caprines might have a more recent common ancestor with cervids than with bovines, and, indeed, the tree produced in our study by the UWPGM algorithm depicts such an ancestry relationship. Nevertheless, the best arrangements of artiodactyl branches with respect to lowering the APSD coefficient placed the caprines closer to bovines than to cervids in agreement with morphological evidence on ruminant relationships.

The joining of carnivores with ungulates before they join the Primates fits established ideas on mammalian phylogeny, for the Palaeocene Condylartha and Carnivora (from which the two ungulate orders, Perissodactyla and Artiodactyla, descended) are traced to late Cretaceous palaeoryctoid insectivores⁷⁷. The marked divergence of the rabbit line from all other mammals is also in agreement with fossil data on mammalian phylogeny⁷⁷. Although joining rat to rabbit at

peptides are identical. Furthermore, the mutational divergence among the catarrhine primates is not nearly as marked as in other comparable taxonomic groups. In this connexion, the fibrinopeptides of the catarrhine primates are among the least rapidly evolving ones in the class Mammalia as revealed by the patristic mutation lengths in Fig. 4.

In addition to the amino-acid sequence data on haemoglobin chains and fibrinopeptides, there are partial sequence data on the erythrocyte enzyme carbonic anhydrase B which can be used to depict cladistic relationships among catarrhine primates⁷⁸. Of the 133 homologous residues examined, chimpanzee and man differ at only one site, whereas orang utan differs from chimpanzee at three sites and from man at four sites. These hominoids differ from cercopithecoids (vervet, irus and rhesus macaques, and baboon) at four to six sites. Thus again the very close genetic relationship between man and chimpanzee is highlighted. The findings from the various sets of amino-acid sequence data support the conclusion drawn from extensive comparative immunodiffusion studies⁷⁹⁻⁸² on primate proteins that in the descent of the

Fig. 4 Mammalian fibrinopeptide phylogenetic tree. 1, Rabbit^{67,68}; 2, rat⁶⁷; 3, *Macaca mulatta*⁶⁹; 4, drill (*Mandrillus leucophaeus*)⁷⁰; 5, vervet (*Cercopithecus aethiops*)⁶⁹; 6, gibbon-1 (3 Gly-numbered from right to left)⁷¹; 7, gibbon-2 (3 Ser)⁷¹; 8, man⁶⁹; 9, chimpanzee⁷²; 10, horse^{67,68}; 11, mule-1^{67,68}; 12, zebra-2⁷³; 13, zebra-1⁷³; 14, donkey⁶⁷; 15, mule-2^{67,68}; 16, pig^{67,68}; 17, camel^{74,75}; 18, llama^{67,68}; 19, vicuna (*Vicugna vicugna*)^{74,75}; 20, reindeer (*Rangifer tarandus*)^{74,75}; 21, mule deer (*Odocoileus hemionus hemionus*)⁷⁴; 22, muntjak (*Muntiacus muntjak*)^{74,75}; 23, sika deer (*Cervus nippon*)^{67,68}; 24, red deer (*Cervus elaphus*)^{67,68}; 25, American elk (*Cervus canadensis*)^{74,75}; 26, pronghorn (*Antilocapra americana*)^{74,75}; 27, sheep^{67,68}; 28, goat^{67,68}; 29, bovine^{67,68}; 30, European bison^{67,68}; 31, Cape buffalo^{67,68}; 32, water buffalo^{67,68}; 33, cat^{67,68}; 34, dog^{67,68}; 35, fox^{67,68}; 36, kangaroo⁷³.



the apex of the tree is not in accord with the idea⁷⁷ from palaeontological evidence that rodents evolved out of the basal Primates, it may be noted that rat fibrinopeptides show less mutational divergence from primate fibrinopeptides than from those of other mammals (Table 4). Furthermore, the globin phylogenetic trees joined mouse to the base of the Primates. A finding at variance with our general knowledge of mammalian phylogeny is the grouping of kangaroo, a marsupial, with the carnivore branch. Fibrinopeptides from additional marsupials need to be sequenced, however, if we are to construct a phylogenetic tree for the relationship between marsupial and placental fibrinopeptides.

In the descent of primate fibrinopeptides, where hominoids (chimpanzee and man) and cercopithecoids (macaque, vervet, drill) branch apart, the gibbons split off as a distinct lineage from the base of the cercopithecoids. No splitting occurs in the terminal descent of man and chimpanzee: their fibrino-

peptides are identical. Furthermore, the mutational divergence among the catarrhine primates is not nearly as marked as in other comparable taxonomic groups. In this connexion, the fibrinopeptides of the catarrhine primates are among the least rapidly evolving ones in the class Mammalia as revealed by the patristic mutation lengths in Fig. 4.

Four to five million year old fossil remains of man's immediate generic ancestor, *Australopithecus*, have recently been found in Pliocene deposits of Ethiopia⁸⁴, and fourteen million year old remains of *Ramapithecus*⁸⁶ (*Kenyapithecus*⁸⁷), interpreted as a lineal ancestor of *Australopithecus*, date from the terminal Miocene of Kenya. Another Kenya hominoid, an eighteen million year old *Dryopithecus* ape, is considered an ancestor of *Gorilla* and to have even then separated from the line to *Ramapithecus* and *Homo*⁸⁸. The hypothesis that

Table 4 Mutation Values from Pairwise Comparisons of Mammalian Fibrinopeptide A and B Sequences

	Rabbit	Rat	Kulatta	Drill	Vervet	Gibbon-1	Gibbon-2	Man	Chimp	Horse	Mule-1	Zebra-2	Zebra-1	Donkey	Mule-2	Pig	Camel	Llama	Vicuna	Reindeer	Mule Deer	Muntjak	Sika Deer	Red Deer	Am. Elk	Pronghorn	Sheep	Goat	Bovine	Eur. Bison	Cape Buffalo	Water Buffalo	Cat	Dog	Fox	Kangaroo	
Rabbit	0																																				
Rat	22	0																																			
Kulatta	20	16	0																																		
Drill	18	15	6	0																																	
Vervet	17	15	3	3	0																																
Gibbon-1	16	12	5	6	3	0																															
Gibbon-2	17	11	4	7	4	1	0																														
Man	18	14	5	8	5	4	3	0																													
Chimp	18	14	5	8	5	4	3	0	0																												
Horse	20	19	15	18	16	14	15	19	19	0																											
Mule-1	20	19	15	18	16	14	15	19	19	0	0																										
Zebra-2	22	22	18	19	19	17	18	22	22	8	8	0																									
Zebra-1	22	22	17	19	18	16	17	21	21	7	7	2	0																								
Donkey	24	22	18	19	19	17	18	22	22	6	6	2	2	0																							
Mule-2	24	22	18	19	19	17	18	22	22	6	6	2	2	0	0																						
Pig	24	26	19	22	19	16	17	22	22	15	15	15	14	14	14	0																					
Camel	19	21	15	19	16	12	13	17	17	14	14	14	13	14	14	13	0																				
Llama	20	20	16	20	17	13	14	18	18	15	15	15	14	15	15	12	1	0																			
Vicuna	20	20	16	20	17	13	14	18	18	15	15	15	14	15	15	12	1	0	0																		
Reindeer	21	26	15	20	18	14	15	19	19	17	17	17	16	17	17	17	14	15	15	0																	
Mule Deer	21	26	15	20	18	14	15	19	19	16	16	18	17	18	18	18	12	13	13	1	0																
Muntjak	23	24	17	20	20	16	17	21	21	18	18	18	18	18	18	16	14	14	14	4	5	0															
Sika Deer	21	22	17	23	21	17	18	22	22	19	19	19	18	19	19	17	14	14	14	6	7	4	0														
Red Deer	21	23	16	22	20	18	19	21	21	20	20	20	19	20	20	18	15	15	15	7	8	5	1	0													
Am. Elk	21	23	16	22	20	18	19	21	21	20	20	20	19	20	20	18	15	15	15	7	8	5	1	0	0												
Pronghorn	19	31	22	26	25	21	22	26	26	21	21	20	22	22	22	25	20	21	21	14	15	15	16	17	17	0											
Sheep	22	26	14	21	19	14	15	20	20	14	14	16	15	16	16	19	16	17	17	11	9	12	14	15	15	18	0										
Goat	22	26	14	21	19	14	15	20	20	14	14	16	15	16	16	19	16	17	17	11	9	12	14	15	15	16	0	0									
Bovine	26	24	19	20	20	17	18	20	20	20	20	22	22	21	21	18	18	18	18	16	16	13	16	15	15	24	22	22	0								
Eur. Bison	24	22	17	19	17	14	15	17	17	19	19	20	19	19	19	17	15	15	15	13	13	12	13	12	12	22	19	19	3	0							
Cape Buffalo	23	19	12	16	14	9	10	14	14	16	16	15	14	14	14	11	12	12	12	11	11	11	12	13	13	19	16	16	5	3	0						
Water Buffalo	25	16	21	19	14	15	19	19	18	18	20	19	19	19	19	17	14	15	15	13	13	14	15	16	16	20	17	17	7	6	2	0					
Cat	23	22	18	19	18	14	15	18	18	23	23	22	21	22	22	17	19	20	20	21	22	22	21	22	22	29	23	23	23	22	15	21	0				
Dog	25	23	18	26	25	22	23	25	25	22	22	20	19	18	18	23	21	22	22	23	24	25	24	25	25	27	23	23	29	27	18	25	19	0			
Fox	25	23	18	26	25	22	23	25	25	23	23	21	20	19	19	24	21	22	22	22	23	25	24	25	25	27	24	24	29	27	18	25	20	2	0		
Kangaroo	20	30	14	18	18	16	17	21	21	25	25	20	19	20	20	24	22	23	23	24	25	27	27	27	27	27	27	25	25	29	27	20	26	22	13	13	0

The half matrix lists minimum numbers of mutations interrelating pairs of fibrinopeptides. Numbers of shared amino-acids vary considerably in mammalian fibrinopeptides. For example, human has thirty amino-acid residues and rhesus has twenty-five residues, all the positions of which are shared by human. The mutation value for the human-rhesus pair presented in the table is obtained from the twenty-five shared amino-acid positions. Cape buffalo has thirty-six residues, only twenty-six positions of which are shared by human. Thus the mutation value for the human-Cape buffalo pair is obtained from these twenty-six shared positions. The alignments for most of the fibrinopeptide sequences can be found in ref. 14. The mutation values are converted into % mutational divergence values for constructing the phylogenetic tree in Fig. 4.

molecular evolution decelerated in higher primates reconciles such palaeontological views with protein findings on the close genetic relationship of the African apes to man. DNA reassociation experiments^{89,90} on nonrepeating polynucleotide sequences (the DNA fraction best suited for cladistic comparisons) further demonstrate the close relationship between *Pan* and *Homo*. The DNA findings emphasize the possibility that steadily increasing generation lengths may have been an important parameter^{79,89,90} in slowing molecular evolution during the past sixty-five million years in the descent of man.

We thank Mr Walter Farris for assistance, Miss Joan Bechtold and Mrs Geraldine Fockler for drawings, and the staff of the Wayne State University Computing Center for help. This work was supported by grants from the US National Science Foundation Systematic Biology and US-Japan Cooperative Science Programs.

Received February 17; revised March 18, 1971.

- ¹ Ingram, V. M., *Nature*, **189**, 704 (1961).
- ² Goodman, M., *Human Biol.*, **33**, 131 (1961).
- ³ Goodman, M., in *Classification and Human Evolution*, 203 (Aldine Press, 1963).
- ⁴ Goodman, M., in *Protides of Biological Fluids*, 70 (Elsevier, Amsterdam, 1965).
- ⁵ Kimura, M., *Nature*, **217**, 624 (1968).
- ⁶ King, J. L., and Jukes, T. H., *Science*, **164**, 788 (1969).
- ⁷ Fitch, W. M., and Margoliash, E., *Science*, **155**, 279 (1967).
- ⁸ Sokal, R., and Sneath, P. H. A., *Principles of Numerical Taxonomy*, 309 (W. H. Freeman, San Francisco, 1963).
- ⁹ Moore, G. W., thesis, Univ. North Carolina (1970).
- ¹⁰ Barnabas, J., Goodman, M., and Moore, G. W., *Comp. Physiol. Biochem.*, **39B**, 455 (1971).
- ¹¹ Braun, V., Crichton, R. R., and Braunitzer, G., *Z. Physiol. Chem.*, **349**, 45 (1968).
- ¹² Braunitzer, G., Buse, G., and Braig, S., *Z. Physiol. Chem.*, **350**, 1477 (1969).
- ¹³ Edmundson, A. B., *Nature*, **205**, 883 (1965).
- ¹⁴ Dayhoff, M. O., *Atlas of Protein Sequence and Structure*, **4** (1969).
- ¹⁵ Rudloff, V., Zelenik, M., and Braunitzer, G., *Z. Physiol. Chem.*, **344**, 284 (1966).
- ¹⁶ Hilse, K., and Braunitzer, G., *Z. Physiol. Chem.*, **349**, 433 (1968).
- ¹⁷ Matsuda, G., Takei, H., Wu, K. C., Mizuno, K., and Shiozawa, T., *Eighth Intern. Cong. Biochemistry*, Abstract, **4** (1970).
- ¹⁸ Von Ehrenstein, G., *Cold Spring Harbor Symp. Quant. Biol.*, **31**, 705 (1966).
- ¹⁹ Hill, R. L., unpublished data taken from *Handbook of Biochemistry* (The Chemical Rubber Company, 1968).
- ²⁰ Rifkin, D. B., Hirsch, D. I., Rifkin, M. R., and Konigsberg, W., *Cold Spring Harbor Symp. Quant. Biol.*, **31**, 715 (1966).
- ²¹ Popp, R. A., *J. Mol. Biol.*, **27**, 9 (1967).
- ²² Matsuda, G., Maita, T., Takei, H., Ota, H., Yamaguchi, M., Miyanchi, T., and Migita, M., *J. Biochem. (Japan)*, **64**, 279 (1968).
- ²³ Zuckerkandl, E., and Schroeder, W. A., *Nature*, **192**, 984 (1961).
- ²⁴ Rifkin, D. B., and Konigsberg, W., *Biochim. Biophys. Acta*, **104**, 457 (1965).
- ²⁵ Braunitzer, G., Gehring-Mueller, R., Hilschmann, N., Hilse, K., Hobom, G., Rudloff, Y., and Wittman-Liebold, B., *Z. Physiol. Chem.*, **325**, 283 (1961).
- ²⁶ Schroeder, W. A., Shelton, J. R., Shelton, J. B., and Cormick, J., *Biochemistry*, **2**, 1353 (1963).
- ²⁷ Kilmartin, J. V., and Clegg, J. B., *Nature*, **213**, 269 (1967).
- ²⁸ Matsuda, G., Gehring-Mueller, R., and Braunitzer, G., *Biochem. Z.*, **338**, 669 (1963).
- ²⁹ Schroeder, W. A., Shelton, J. R., Shelton, J. B., Robberson, B., and Babin, D. R., *Arch. Biochem. Biophys.*, **120**, 1 (1967).
- ³⁰ Huisman, T. H. J., Brandt, G., and Wilson, J. B., *J. Biol. Chem.*, **243**, 3637 (1968).
- ³¹ Huisman, T. H. J., Dozy, A. M., Wilson, J. B., Effremov, G. D., and Vaskov, B., *Biochim. Biophys. Acta*, **160**, 467 (1968).
- ³² Beale, D., Lehman, H., Drury, A., and Tucker, E. M., *Nature*, **209**, 1099 (1966).
- ³³ Wilson, J. B., Brandt, G., and Huisman, T. H. J., *J. Biol. Chem.*, **243**, 3687 (1968).
- ³⁴ Schroeder, W. A., Shelton, J. R., Shelton, J. B., Cormick, J., and Jones, R. T., *Biochemistry*, **2**, 992 (1963).
- ³⁵ Air, G. M., and Thomson, E. O. P., *Austral. J. Biol. Sci.*, **22**, 1437 (1969).
- ³⁶ Rifkin, D. B., Rifkin, M. R., and Konigsberg, W., *Proc. US Nat. Acad. Sci.*, **55**, 586 (1966).
- ³⁷ Braunitzer, G., Best, J. S., Flamm, U., and Schrank, B., *Z. Physiol. Chem.*, **347**, 207 (1966).
- ³⁸ Boyer, S. H., Crosby, E. F., Thurmon, T. F., Noyes, A. N., Fuller, G. F., Leslie, S. E., and Sheppard, M. K., *Science*, **116**, 1428 (1969).
- ³⁹ Matsuda, G., Maita, T., Ota, H., Tachiwaka, I., Tanaka, Y., Araya, A., and Nakashima, Y., *Intern. J. Protein Res.*, **3**, 41 (1971).
- ⁴⁰ Ingram, V. M., and Stretton, A. O. W., *Biochim. Biophys. Acta*, **63**, 20 (1962).
- ⁴¹ Smith, D. B., *Canad. J. Biochem.*, **46**, 825 (1968).
- ⁴² Babin, D. R., Schroeder, W. A., Shelton, J. R., Shelton, J. B., and Robberson, B., *Biochemistry*, **5**, 1297 (1966).
- ⁴³ Wilson, J. B., Edwards, W. C., McDaniel, M., Dobbs, M. M., and Huisman, T. H. J., *Arch. Biochem. Biophys.*, **115**, 385 (1966).
- ⁴⁴ Schroeder, W. A., Shelton, J. R., Shelton, J. B., Robberson, B., and Babin, D. R., *Arch. Biochem. Biophys.*, **120**, 124 (1967).
- ⁴⁵ Huisman, T. H. J., Dasher, G. A., Moretz, W. H., Dozy, A. M., and Wilson, J. B., *Biochem. J.*, **107**, 745 (1968).
- ⁴⁶ Boyer, S. H., Hathaway, P., Pascasio, F., Orton, C., Bordley, J., and Naughton, M. A., *Science*, **153**, 1539 (1966).
- ⁴⁷ Huisman, T. H. J., Adams, H. R., Dimock, M. O., Edwards, W. E., and Wilson, J. B., *J. Biol. Chem.*, **242**, 2534 (1967).
- ⁴⁸ Huber, R., Epp, O., and Formanck, H., *J. Mol. Biol.*, **42**, 59 (1969).
- ⁴⁹ Briehl, R. W., *J. Biol. Chem.*, **238**, 2361 (1963).
- ⁵⁰ Balani, A. S., and Barnabas, J., *Nature*, **205**, 1019 (1965).
- ⁵¹ Balani, A. S., Ranjekar, P. K., and Barnabas, J., *Comp. Physiol. Biochem.*, **24**, 809 (1968).
- ⁵² Huisman, T. H. J., Wilson, J. B., and Adams, H. R., *Arch. Biochem. Biophys.*, **121**, 528 (1967).
- ⁵³ Ranjekar, P. K., and Barnabas, J., *Indian J. Biochem.*, **6**, 1 (1969).
- ⁵⁴ Barnicot, N. A., and Huehns, E. R., *Nature*, **215**, 1485 (1967); Wade, P. T., Skinner, A. F., and Barnicot, N. A., *Protides in Biological Fluids*, 263 (Pergamon, London, 1970).
- ⁵⁵ Oliver, E., and Kitchen, H., *Biochim. Biophys. Acta, Res. Commun.*, **31**, 749 (1968).
- ⁵⁶ Hilse, K., and Popp, R. A., *Proc. US Nat. Acad. Sci.*, **61**, 930 (1968).
- ⁵⁷ Kunkel, H. G., and Wallenius, G., *Science*, **122**, 288 (1955).
- ⁵⁸ Kunkel, H. G., Ceppellini, R., Mueller-Eberhard, V., and Wolf, J., *J. Clin. Invest.*, **36**, 1615 (1957).
- ⁵⁹ Huisman, T. H. J., Reynolds, C. A., Dozy, A. M., and Wilson, J. B., *J. Biol. Chem.*, **240**, 2455 (1965).
- ⁶⁰ Hamilton, W. J., Boyd, J. D., and Mossman, H. W., *Human Embryology* (Williams and Wilkins, Baltimore, 1952).
- ⁶¹ Kimura, M., *Proc. US Nat. Acad. Sci.*, **63**, 1181 (1969).
- ⁶² Buettner-Janusch, J., and Buettner-Janusch, V., *Amer. J. Phys. Anthropol.*, **33**, 73 (1970).
- ⁶³ Ohno, S., *Evolution by Gene Duplication* (Springer, Berlin, 1970).
- ⁶⁴ Buettner-Janusch, J., and Hill, R. W., in *Evolving Genes and Proteins*, 167 (Academic Press, London and New York, 1965).
- ⁶⁵ LeGros Clark, W. E., *The Antecedents of Man* (Edinburgh University Press, Edinburgh, 1959).
- ⁶⁶ Buettner-Janusch, J., Buettner-Janusch, V., and Mason, G. A., *Arch. Biochem. Biophys.*, **133**, 164 (1969).
- ⁶⁷ Blombäck, B., Blombäck, M., and Grondahl, N. J., *Acta Chem. Scand.*, **19**, 1789 (1965).
- ⁶⁸ Blombäck, B., Blombäck, M., Grondahl, N. J., and Holmberg, E., *Arkiv. Kemi.*, **25**, 411 (1966).
- ⁶⁹ Blombäck, B., Blombäck, M., Grondahl, N. J., Guthrie, C., and Hinton, M., *Acta Chem. Scand.*, **19**, 1788 (1965); Blombäck, B., Blombäck, M., and Edman, B., *Biochim. Biophys. Acta*, **115**, 371 (1966).
- ⁷⁰ Doolittle, R. F., Glasgow, C., and Mross, G. A., *Biochim. Biophys. Acta*, **175**, 217 (1969).
- ⁷¹ Mross, G. A., Doolittle, R. F., and Roberts, B. F., *Science*, **170**, 468 (1970).
- ⁷² Doolittle, R. F., and Mross, G. A., *Nature*, **225**, 643 (1970).
- ⁷³ Blombäck, B., and Blombäck, M., in *Chemotaxonomy and Serotaxonomy*, 3 (Academic Press, London and New York, 1968).
- ⁷⁴ Doolittle, R. F., Schubert, D., and Schwartz, S. A., *Arch. Biochem. Biophys.*, **118**, 456 (1967).
- ⁷⁵ Mross, G. A., and Doolittle, R. F., *Arch. Biochem. Biophys.*, **122**, 674 (1967).
- ⁷⁶ Doolittle, R. F., and Blombäck, B., *Nature*, **202**, 147 (1964).
- ⁷⁷ McKenna, M. C., *Ann. NY Acad. Sci.*, **167**, 217 (1969).
- ⁷⁸ Tashian, R. E., and Stroup, S. R., *Biochem. Biophys. Res. Commun.*, **41**, 1457 (1970).
- ⁷⁹ Goodman, M., *Human Biol.*, **34**, 104 (1962).
- ⁸⁰ Goodman, M., *Ann. NY Acad. Sci.*, **102**, 219 (1962).
- ⁸¹ Goodman, M., *Human Biol.*, **35**, 377 (1963).
- ⁸² Goodman, M., *Primates in Med.*, **1**, 10 (1968).
- ⁸³ Darwin, C., *The Descent of Man and Selection in Relation to Sex* (Appleton, New York, 1871).
- ⁸⁴ Patterson, B., Behrensmeier, A. K., and Sill, W. D., *Nature*, **226**, 918 (1970).
- ⁸⁵ Howell, F. C., *Nature*, **223**, 1234 (1969).
- ⁸⁶ Simons, E. L., *Nature*, **221**, 448 (1969).
- ⁸⁷ Leakey, L. S. B., *Nature*, **213**, 155 (1967).
- ⁸⁸ Pilbeam, D. R., *Peabody Mus. Bull. Yale*, **31** (1969).
- ⁸⁹ Kohne, D. E., *Quart. Rev. Biophys.*, **3**, 327 (1970).
- ⁹⁰ Kohne, D. E., Chison, J. A., and Hoyer, B. H., *Carnegie Inst. Yr Book*, **69**, 488 (1971).

LETTERS TO NATURE

PHYSICAL SCIENCES

Krypton-85 in the Troposphere

KRYPTON-85 produced by fission in nuclear reactors is at present the most abundant man-made radioisotope in the troposphere. Its activity in air was 33 d.p.m. m^{-3} air by the end of 1969 and it continues to increase. In this communication we report recent measurements of the ^{85}Kr activity in samples from air liquefaction plants at Oberhausen and Duisburg in West Germany.

The raw krypton samples were purified by removal of the oxygen in a magnesium furnace, and their activity was measured in a small gas proportional counter, of sensitive volume 11 cm^3 , surrounded by a lead shield 10 cm thick; a plastic scintillator was used as an anticoincidence counter. The counting efficiency was determined from other measurements on three ^{85}Kr samples in ^{14}C and T counters whose efficiencies were known. All samples were analysed more than four months after collection and the contributions of ^{133}Xe and ^{222}Rn (half-lives of 5.65 days and 3.82 days respectively) could be neglected. The amount of Xe in the purified krypton samples ($\sim 10\%$) was measured mass spectrometrically and the appropriate correction made. The only other correction was that for radioactive decay since collection. The mean standard deviation of the ^{85}Kr measurement is $\pm 2.5\%$.

The data are presented in Fig. 1, together with data obtained previously in our laboratory by Schölch and already published in part^{1,2}. The earlier values had to be lowered by about 5 to 9% to correct a calibration error and to compensate for radioactive decay. The increase of the atmospheric ^{85}Kr activity during the past decade is apparently linear and a least squares fit of a straight line gives an average net increase

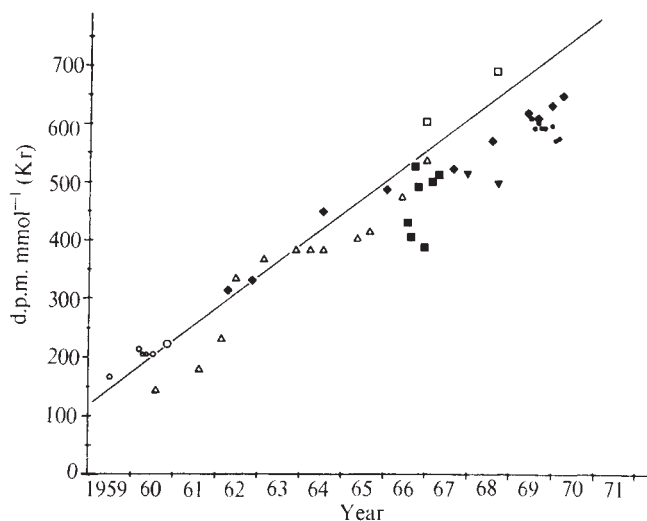


Fig. 2 ^{85}Kr activities reported at various locations by various authors. $\circ$, Data from West Germany⁷; $\triangle$, data from France³; $\square$, data from Lyon, France³. The solid symbols represent measurements from North America^{3,4}: $\blacklozenge$, Cleveland, Ohio; $\blacksquare$, Essington, Pennsylvania; $\blacktriangle$, Ontario; $\bullet$, Chester, West Virginia.

of 55 d.p.m. $\text{mmol}^{-1}(\text{Kr})\text{ yr}^{-1}$. The actual annual production of ^{85}Kr is, of course, larger than this because of the radioactive decay. Because the total tropospheric content of ^{85}Kr and thus the loss by radioactive decay increased linearly, the actual release of ^{85}Kr activity must have increased linearly with time by $(1+t/\tau)$, from 64.5 d.p.m. $\text{mmol}^{-1}(\text{Kr})\text{ yr}^{-1}$ in 1959 to 99.5 d.p.m. $\text{mmol}^{-1}(\text{Kr})\text{ yr}^{-1}$ in 1969. t is the time counted from late 1956 and τ is the decay time of ^{85}Kr , 15.53 yr.

In Fig. 2 the ^{85}Kr measurements of other authors are compared with the fitted line. Although the individual data show a considerable scatter, even where monthly samples have been collected^{2,3}, the overall agreement is reasonably good and indicates that systematic differences between the various laboratories due to calibration errors are not too serious. The observed variations in the atmospheric ^{85}Kr concentration are probably real and are presumably caused by the discontinuous release of ^{85}Kr from fuel reprocessing plants and variations in horizontal and vertical mixing. A seasonal variation of the ^{85}Kr activity, which would be expected if ^{85}Kr were produced chiefly by nuclear explosions, cannot be detected above the noise level.

The data of Shuping *et al.*^{3,4} from various stations of the North American continent in the years 1967 to 1969 fall below the fitted line of Fig. 2. As data from Lyon, France, measured by Shuping during the same period fall above the line, the apparent displacement cannot be explained by differences in calibration between the laboratories. In fact the average ^{85}Kr activity during 1968 and 1969 (including December 1967) was 587 ± 9.2 for the North American stations and 668 ± 29 for the Oberhausen and Duisburg samples, including the one sample from Lyon³. The mean standard deviations were calculated taking into account the systematic increase of 55 d.p.m. $\text{mmol}^{-1}(\text{Kr})\text{ yr}^{-1}$. The difference is 81 ± 30 , which seems significant.

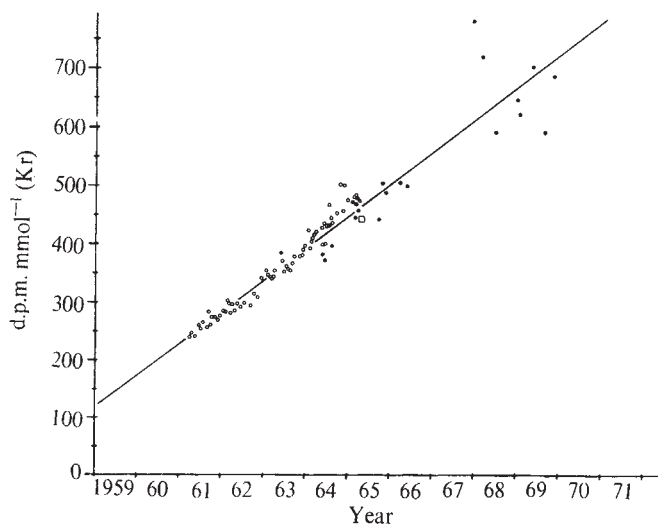


Fig. 1 ^{85}Kr activity in surface air at Duisburg and Oberhausen, Ruhr valley. $\bullet$, Recent measurements; $\circ$, previous measurements from our laboratory; $\square$, a sample distributed by the plant in Munich but probably collected at Oberhausen and measured by Shuping *et al.*³. The straight line is a least squares fit to the experimental data.

The higher ^{85}Kr concentrations in the Ruhr valley could be derived from local sources. The large scatter of the samples from the Ruhr valley in 1968 and 1969, which is much larger than that of previous years (Fig. 1), also suggests contamination by local sources.

Finally the tropospheric burden of ^{85}Kr can be estimated from the present data. Using Pannetier's data⁵ on meridional and height distribution of ^{85}Kr we obtain 9.65×10^{26} atoms ^{85}Kr by 1969. Assuming a linear increase of the tropospheric ^{85}Kr activity starting from zero at the end of 1956, this amount corresponds to a total ^{85}Kr production of 1.36×10^{27} atoms ^{85}Kr , which would correspond to the fission of 182 tons of ^{235}U (fission yield for thermal neutrons 0.293%, ref. 6).

The samples were provided by W. Stich of the Linde AG, Munich. The National Center for Atmospheric Research is sponsored by the National Science Foundation.

J. SCHRÖDER
K. O. MÜNNICH

II Physikalisches Institut,
C-14 Laboratorium,
Universität Heidelberg

D. H. EHHALT

National Center for Atmospheric Research,
Boulder, Colorado 80302

Received August 16, 1971.

¹ Ehhalt, D. H., Münnich, K. O., Roether, W., Schölch, J., and Stich, W., *J. Geophys. Res.*, **68**, 3817 (1963).

² Ehhalt, D. H., Münnich, K. O., Roether, W., Schölch, J., and Stich, W., *Third United Nations International Conference on the Peaceful Uses of Atomic Energy (A/CONF. 28/P/541, May 1964)*.

³ Shuping, R. E., Phillips, C. R., and Moghissi, A. A., *Anal. Chem.*, **41**, 2082 (1969).

⁴ Shuping, R. E., Phillips, C. R., and Moghissi, A. A., *Radiolog. Health Data and Repts*, **11**, 671 (1970).

⁵ Pannetier, R., *Rapport CEA-R-3591* (Saclay, 1968).

⁶ Katcoff, S., *Nucleonics, Data Sheet No. 24*, **16**, 78 (1958).

⁷ Griesser, O., and Sittkus, A., *Z. Naturforsch.*, **16**, 620 (1961).

Amino-acid Synthesis from Gases detected in Interstellar Space

Fox and Windsor¹ have synthesized amino-acids by heating a solution of formaldehyde, ammonia and water to 185° C and they interpreted their results in a cosmochemical context. We now report that reaction products from different combinations of ammonia, methanol vapour, formic acid vapour and formaldehyde react to produce amino-acids when exposed to ultraviolet radiation. Because ammonia², methanol³, formic acid⁴ and formaldehyde⁵ have been identified in interstellar space, our finding suggests that amino-acids can be formed without water in interstellar space. Also, in spite of the absence of conclusive evidence of the presence of any biomolecules indigenous to the lunar surface, our findings suggest that amino-acids can be formed on the Moon. Although the conditions of our experiments differed from those in interstellar space and on the Moon, we believe that our findings have implications for interstellar chemistry.

The combinations of gases used in our experiments were ammonia, methanol and formaldehyde, and ammonia, methanol and formic acid. The ammonia was obtained from a cylinder of the compressed gas and we generated the formaldehyde gas by heating paraformaldehyde in a flask; the methanol and formic acid vapours were formed by heating the liquids in flasks. Glass tubes led the gases from the generating flasks to a 200 ml. 'Kimax' spherical flask in which the gases reacted.

All chemicals used were anhydrous because we intended to conduct the experiments without water. We also flushed out the mixing flask with ammonia before the two experimental runs to eliminate any residual water vapour. To prevent

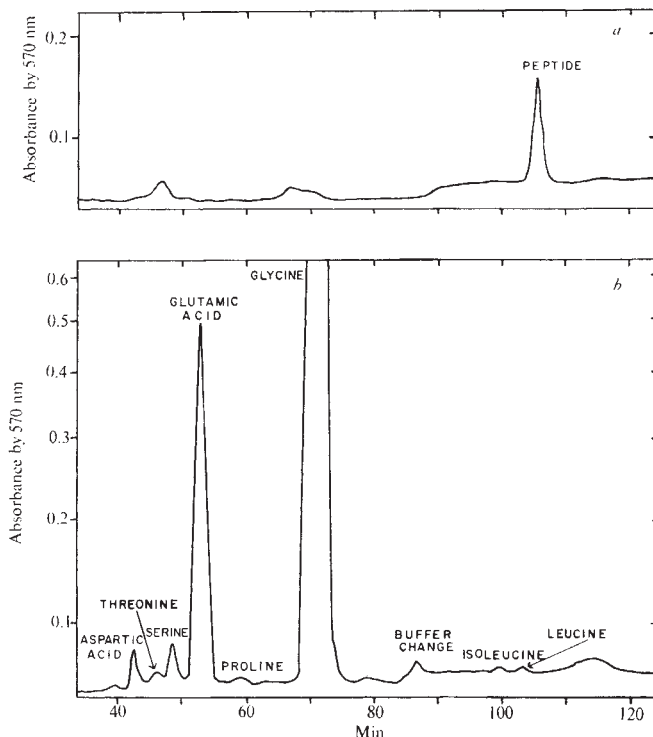


Fig. 1 Amino-acid analysis ion exchange chromatograms. *a*, Unhydrolysed, and *b*, hydrolysed samples (K) of a radiation product of ammonia, methanol vapour and formaldehyde.

condensation of unreacted methanol and formic acid during mixing of the gases, we maintained the temperature of the mixing flask at about 115° C by directing four sunlamps on it (each 275 W) during both runs.

The gases were slowly introduced into the mixing flask and after 45 min the gas flows were stopped and the mixing flask was immediately sealed with a glass stopper.

The ammonia, methanol and formaldehyde reacted to yield 14 g of a pale yellow liquid, and the ammonia, methanol and formic acid yielded 19 g of a colourless liquid. We made no analyses of the reaction products immediately after mixing because our earlier experiments indicated that the formation of amino-acids from reaction products required exposure to ultraviolet radiation.

The flasks containing the reaction products were exposed to ultraviolet radiation from a 275 W sunlamp situated 60 cm below the flasks. The temperature at the bottom of the flasks remained at 35° C.

The liquid produced by mixing ammonia, methanol and formaldehyde started to crystallize after exposure to ultraviolet radiation for 2 days. The other reaction product started to crystallize after 7 days.

Negative tests for amino-acids indicated that the substance produced by mixing ammonia, methanol and formic acid had to be exposed to ultraviolet radiation for 25 days before amino-acids formed. The product yielded by mixing ammonia, methanol and formaldehyde was first analysed for amino-acids after exposure to ultraviolet radiation for 3 days and the analysis was positive.

The analyses for amino-acids were carried out by the method described by Moore *et al.*⁶, Spackman *et al.*⁷, Moore and Stein⁸, and Granberg *et al.*⁹. The instrument used was a Beckman-Spinco model 120B amino-acid analyser. The samples were hydrolysed by treatment with 6 N HCl at 110° C under vacuum in sealed tubes for 24 h. Hydrolysis of the radiation reaction product of ammonia, methanol and formaldehyde yielded chiefly glycine and glutamic acid (Fig. 1). Relatively small amounts of aspartic acid, threonine, serine, proline, isoleucine and leucine were, however, also present.

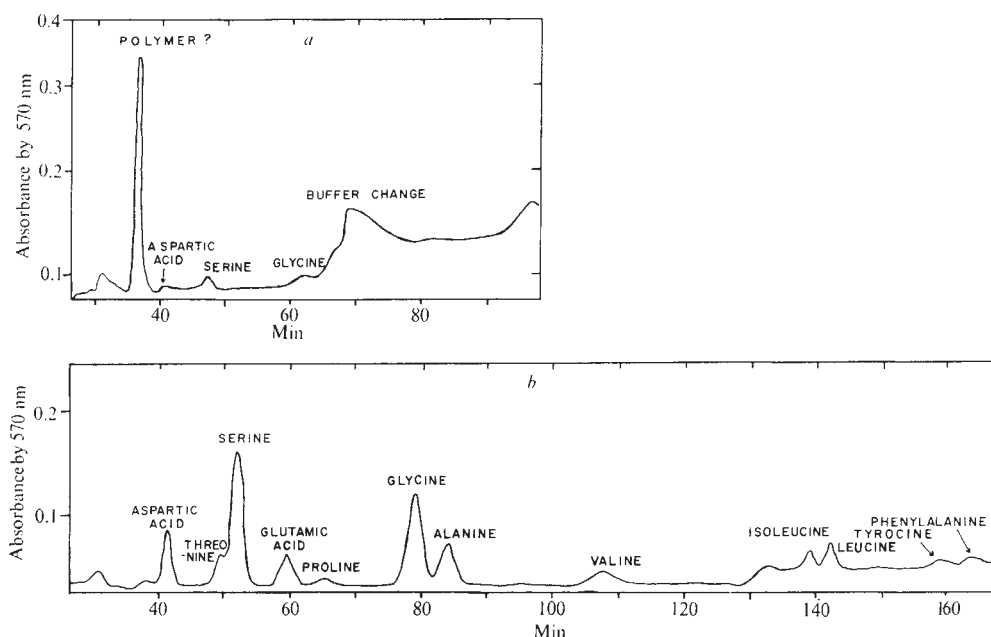


Fig. 2 Amino-acid analysis ion exchange chromatograms. *a*, Unhydrolysed, and *b*, hydrolysed samples (M) of a radiation reaction product of ammonia, methanol vapour and formic acid vapour.

The radiation reaction product of ammonia, methanol and formic acid yielded a larger number of amino-acids after hydrolysis. These included aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine and phenylalanine (Fig. 2).

On hydrolysis, 50 mg of the radiation reaction product of ammonia, methanol and formaldehyde produced 0.135 mg of amino-acids—a yield of 0.27%. On the other hand, 50 mg of the radiation reaction product of ammonia, methanol and formic acid gave 0.07 mg of amino-acids—a yield of 0.14%.

The amino-acids produced by the ammonia-methanol-formic acid system are similar to those produced by Fox and Windsor¹ on heating formaldehyde, ammonia and water, and to those produced by Pavlovskaya and Pasynski¹⁰ by ultraviolet radiation of ammonium chloride, formaldehyde and water.

The experimental procedure eliminated the possibility of accidental introduction of amino-acids as contaminants. The ammonia was provided from a cylinder of the compressed gas and thus it could not contain amino-acids—which are either non-volatile or sublime at temperatures greater than that at which the ammonia was drawn from the cylinder. By heating the paraformaldehyde, formic acid and methanol in separate flasks and introducing only the vapours into the mixing flask we avoided contamination by the non-volatile amino-acids. On the other hand, the relatively low temperature (115° C) at which the flasks were maintained precluded the introduction of those amino-acids which sublime, because they sublime at fairly high temperatures. We also tested each of the substances from which the vapours were generated; no amino-acids were found.

The chromatogram of the unhydrolysed sample (Fig. 1) indicates that ultraviolet radiation of the reaction product of ammonia, methanol and formaldehyde produced a peptide. The high peak shown in the chromatogram (Fig. 2) of the unhydrolysed sample of the other radiation reaction product indicates that a polymer might have formed. In this chromatogram as well as in the chromatogram of the hydrolysed sample shown in Fig. 1, the artefact peak due to a change in the eluting buffer is clearly marked.

A microbiuret test for peptides was positive for the radiation reaction product of ammonia, methanol and formaldehyde. The test of the other radiation reaction product was negative, suggesting that the polymer is the "HCN polymer" described by Labadie *et al.*¹¹, rather than polyamino-acid.

As established by mass spectrographic analyses the radiation

reaction product of ammonia, methanol and formaldehyde yielded hexamethylenetetramine, but the other reaction product did not. The production of hexamethylenetetramine indicates that some water was formed during the reaction.

The facts that one of the reactions yielding amino-acids took place in the absence of water and that we only used gases suggest that perhaps some rethinking is needed about the kind of chemical reactions possible in interstellar conditions. The absence of water in our experiments strongly suggests that compounds which play an important role in the origin of life may form in interstellar space and that such compounds as amino-acids may form on the Moon.

Richard Granberg carried out the amino-acid analyses. We thank Kenneth King who suggested these experiments and M. Ewing, S. Moore, J. Oro, P. B. Hamilton, G. M. Sharma, S. J. Rasool, P. Thaddeus, D. Horn, N. T. Lui, B. Horn and B. Ericson-Moody for help. We also thank Janet Wollin for assistance. The research was supported by the US National Science Foundation.

GOESTA WOLLIN
DAVID B. ERICSON

Lamont-Doherty Geological Observatory,
Columbia University,
Palisades, New York 10964

Received May 28; revised September 14, 1971.

- ¹ Fox, S. W., and Windsor, C. R., *Science*, **170**, 984 (1970).
- ² Cheung, A. C., Rank, D. M., Townes, C. H., Thornton, D. D., and Welch, W. J., *Phys. Rev. Lett.*, **21**, 1701 (1968).
- ³ Gottlieb, C. A., Ball, J. H., and Lilley, A. E., *Astrophys. J.* (in the press).
- ⁴ Gottlieb, C. A., Ball, J. H., Lilley, A. E., and Zuckerman, B., *Astrophys. J.* (in the press).
- ⁵ Snyder, L. E., Buhl, D., Zuckerman, B., and Palmer, P., *Phys. Rev. Lett.*, **22**, 679 (1969).
- ⁶ Moore, S., Spackman, D. H., and Stein, W. H., *Ann. Chem.*, **30**, 1185 (1958).
- ⁷ Spackman, D. H., Stein, W. H., and Moore, S., *Ann. Chem.*, **30**, 1190 (1958).
- ⁸ Moore, S., and Stein, W. H., *Amino Acid Analysis, Methods in Enzymology*, **6**, 819 (1963).
- ⁹ Granberg, R., Walsh, K. W., and Bradshaw, R. A., *Anal. Biochem.*, **30**, 454 (1969).
- ¹⁰ Pavlovskaya, T. E., and Pasynski, A. G., in *The Origin of Life on the Earth* (edit. by Oparin, A. I., Pasynski, A. G., Braunsstein, A. E., and Pavlovskaya, T. E.), 151 (Pergamon, London, 1959).
- ¹¹ Labadie, M., Jensen, R., and Neuzil, E., *Biochim. Biophys. Acta*, **165**, 525 (1968).

High Precision Charged Particle Detector using Noble Liquids

We agree wholeheartedly with P. Rice-Evans¹ and the leading article in *Nature*² that there is a need for higher resolution particle detectors at the new synchrotrons. Following a suggestion by one of us³, we are working towards the development of a thin multi-conductor chamber filled with a noble liquid. Our initial efforts with liquid argon have been described in several NAL Summer Study reports⁴ and the successful operation of a liquid xenon proportional counter is the subject of a recent article⁵.

We shall briefly summarize what has been learned so far.

First, a liquid xenon proportional chamber with an 8 mm diameter cathode and a single 4 μm anode detects charged particles with an efficiency of nearly 100%. The pulse rises in less than 150 ns and is typically 0.15 pCi in size. (Using liquid argon, all our chambers have been sensitive only at scattered points along the wire.)

Second, the electric field necessary for electron avalanche is $\sim 2 \text{ MV cm}^{-1}$. (Consequently the central wire must be the anode in order to avoid field emission.)

Third, for the detection of ionization pulses (liquid gain = 1) produced by a movable collimated source of alpha particles, a 700 μm thick chamber with five wires spaced 25 μm apart has a spatial accuracy better than 15 μm r.m.s.

Fourth, a series of parallel conducting strips mounted on a substrate is capable of inducing electron multiplication.

Fifth, severe electronegative contamination of liquid xenon (due to unknown impurities) can occur even when the content of oxygen and nitrogen is held below 0.1 p.p.m.⁶. Many of our purification techniques are described in ref. 6.

We are now making efforts to improve our control over electronegative impurities, to show that a series of substrate mounted conductors has high precision in the avalanche mode and to develop a practical readout scheme.

Although the liquid xenon multi-conductor chamber provides accurate spatial information in its "thin" form, it also provides efficient, rapid X-ray and γ -ray detection in its "thick" form. The thin form should prove invaluable in the field of high-energy and cosmic-ray physics, and the thick form holds equally great promise in the field of radiology.

This work was done under the auspices of the US Atomic Energy Commission.

STEPHEN E. DERENZO
GERARD SMADJA
ROBERT G. SMITS
HAIM ZAKLAD
LUIS W. ALVAREZ

Lawrence Berkeley Laboratory,
University of California,
Berkeley, California 94720

RICHARD A. MULLER

Space Science Laboratory,
University of California,
Berkeley, California 94720

Received October 1, 1971.

Mechanism of Oil Droplet Fragmentation in High Pressure Homogenizers

THE process of subdividing the relatively large polydisperse oil globules of a coarse oil-in-water emulsion into a large number of smaller globules of narrow size range is termed "homogenization". In a well homogenized emulsion the globules are so small that they have little tendency to rise and separate out; this is a desirable property of many emulsions of technical importance.

One of the most effective types of homogenizing machine utilizes a high pressure pump and a valve of the drop and lift type (Fig. 1a); pumping the coarse primary emulsion to the centre of the seating lifts the spring loaded valve and the emulsion escapes radially through the slit aperture to emerge with its oil phase finely dispersed. Machines with triple-piston pumps to minimize cyclic pressure fluctuations are used extensively in, for example, the dairy industry, for homogenizing milk, cream, ice-cream mix and other products¹. A pressure drop of up to 250 kg cm^{-2} (3,500 pound inch^{-2}) or more may be applied across the valve and, the higher the pressure is, the smaller are the resulting oil globules. The fat globules in whole milk, which form a phase volume of about 0.035, have diameters up to about 10 μm with an average near 3.0 μm . Their subdivision into globules no larger than 1 μm may be accomplished readily with the homogenizer provided that the processing temperature is such that the fat is molten.

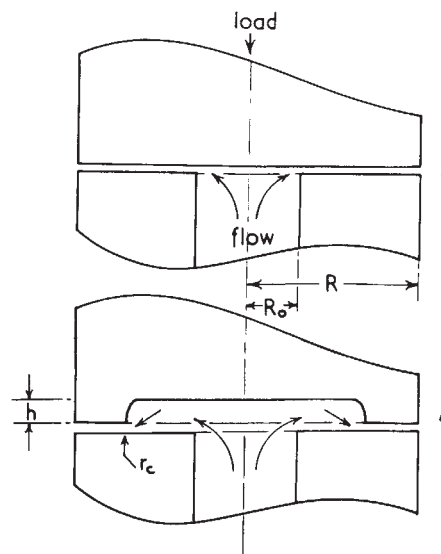


Fig. 1 a, Cross-section of simple flat-faced valve and seating; b, modified valve, to reduce the Reynolds number at r_c , the critical region for globule break-up.

In spite of the effectiveness of the high pressure machine the physical principles underlying the comminution process are obscure. I wish to report experimental data which favour one particular mechanism of droplet fragmentation. Past studies of the action of the piston homogenizer arose chiefly from the interest in homogenizing milk and my discussion is in the same context.

The lack of experimental evidence has precluded the acceptance of any one of the several theories² purporting to explain the physical principles underlying the homogenization of milk. Globule disruption within the valve has been variously thought to result from several effects or combinations of them. These are a shearing action within the milk itself, and between the milk and faces of the valve and its seat^{3,4}; impact of the milk against the side walls of the valve^{5,6}; changes in the milk

¹ Rice-Evans, P., *Nature*, **232**, 625 (1971).

² *Nature*, **232**, 599 (1971).

³ Alvarez, L. W., *Group A Physics Note 672* (University of California Lawrence Radiation Laboratory, 1968).

⁴ Derenzo, S. E., et al., *National Accelerator Laboratory, Batavia, Illinois, Summer Study reports*, SS-154 (1969) and SS-181 (1970).

⁵ Muller, R. A., Derenzo, S. E., Smadja, G., Smith, D. B., Smits, R. G., Zaklad, H., and Alvarez, L. W., *Phys. Rev. Lett.*, **27**, 532 (1971).

⁶ Zaklad, H., thesis, (Lawrence Radiation Laboratory Report, UCRL-20690 (1971)).

velocity coupled with turbulence⁷; forces of cavitation⁸⁻¹⁰ and, more recently, principally turbulence within the milk¹¹.

Some of these possibilities are questionable in the light of experimental observations. The finding that homogenization is almost complete soon after the milk enters the valve slit and before traversing the chief part of the valve radius⁹ is evidence against the effectiveness of both impact forces and shearing between valve faces. Also claims that cavitational forces are responsible are not in accord with experiments which showed that effective homogenization still occurred when calculations indicated that cavitation was unlikely to be taking place in the valve¹².

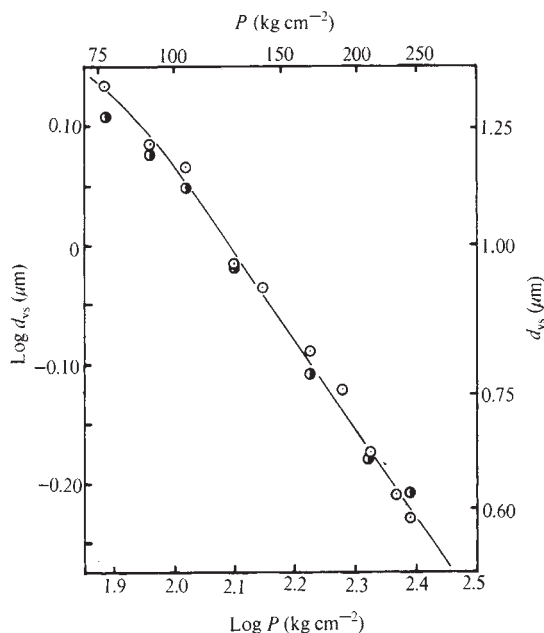


Fig. 2 The relationship between the mean fat globule diameter d_v , and the homogenizing pressure P at a processing temperature of 50° C. ○, Milk, normal flow rate of 100 l. h⁻¹, simple flat-faced valve (Fig. 1a), turbulent flow; ●, milk/glycerol mixture, flow rate 29.9 l. h⁻¹, valve with recessed face (Fig. 1b), laminar flow at the critical radius for globule break-up.

The results from some of my own recent experiments support the conclusion that, in particular, shattering effects, shearing between valve faces and cavitational forces, all of which can occur to varying degree in a valve, have no significant effect on fat globule break-up. Of the remaining possibilities the effects of turbulence are of considerable interest because it has often been supposed that a turbulent flow aids homogenization. Recently, heavy turbulence has been reputed to be the principal effect involved¹¹ because an experimental correlation between the homogenizing pressures used and the mean globule sizes produced seemed to be explained by invoking Kolmogoroff's statistical theories of turbulence. If this were so, it would be expected that laminar flow, rather than the customary turbulent flow, through the valve would result in little or no fragmentation of globules. This has been investigated using a small capacity 3-piston machine (100 l. h⁻¹) (Rannie, Copenhagen, Denmark) in experiments with milk. A temperature of 50° C was chosen for the processing to ensure that the milk fat (melting point ≈ 40° C) would be molten. The degree of homogenization or globule dispersion was assessed by turbidimetric determinations^{13,14} of the mean volume-surface diameter $d_{vs}(= \Sigma nd^3 / \Sigma nd^2)$ of the homogenized milk-fat globules.

Homogenizing milk at different pressures with the simple full-faced valve (Fig. 1a) showed that d_{vs} values, and hence the

kinetics of the globule fragmentation, were reproducible. Typical results are plotted in Fig. 2a and show an approximately linear log/log relation^{13,14}. The effect of increasing the pressure P (which produces smaller globules) and keeping the flow rate Q constant is to produce an increased emulsion velocity through the slit by a reduction in the valve lift, t . Clearly, the degree of globule dispersion is a function of this velocity. Calculations using approximate friction coefficients indicate that, for this machine, t decreases from 50 to 20 or 15 μm as P increases from roughly 10 to 300 kg cm⁻². The velocity at the slit entrance can be high; taking $t=20$ μm, $Q=100$ l. h⁻¹, $r=0.15$ cm, equation (1) gives $v=147$ m s⁻¹.

For a given flow rate Q and viscosity ν of the emulsion, the Reynolds number Re at any radius r of the valve face is independent of both the total pressure drop P across the valve and the width t of the slit between the valve faces. This follows from the form of the equation of continuity which, taking v_r as the mean fluid velocity, is

$$Q = 2\pi r t v_r \quad (1)$$

and which combined with $Re = v_r t / \nu$ gives the relation

$$Re = \frac{Q}{2\pi r \nu} \quad (2)$$

At the entrance to the slit, where $r = R_0 = 0.15$ cm for the valve in question, the Re defined by equation (2) is at a maximum (about 3,000) for milk at 50° C flowing at the normal rate of 100 l. h⁻¹, falling to about 800 at $R=0.55$ cm, the exit radius. The results of recent experiments (L. W. P., to be published) indicate that flow conditions would certainly be regarded as turbulent for these values of Re , but values of $Re \lesssim 250$ would be needed for laminar flow to be established within the valve slit.

Laminar conditions were achieved experimentally as follows. A circular recess of radius 0.4 cm was machined in the upper valve surface (Fig. 1b), leaving only an outer annulus of metal 0.15 cm wide to bear on the seat. The depth h of the recess was calculated to be such that the emulsion would suffer virtually no change in velocity immediately upon turning into the recess from the central conduit. This was assured by making $h = R_0/2 = 0.075$ cm. Radial flow of the emulsion with diminishing velocity would ensue up to the leading edge of the annular ridge, and it would then accelerate into the slit, to an extent depending on the valve lift, before finally emerging. This valve geometry produced a degree of homogenization of milk ($Q=100$ l. h⁻¹) equal to that attained at the same pressure with wholly plane surfaces. Levelling-off the annular ridge but maintaining a separation as large as h would be equivalent to operating a completely flat valve at $P \approx 0$, and no homogenization would take place. It is certain, therefore, that globule break-up occurred at the ridge where, at the rate of flow in question, Re was about 1,125 ($r=0.4$ cm). A lowering of the value of Re at this critical radius was then achieved by reducing Q to 28.9 l. h⁻¹ (that is, to about one-third of the usual rate), thus producing a transitional state of flow ($Re \approx 320$) in the slit entrance. Such a change in conditions did not, however, impair the homogenizing effect of the valve and results were obtained for milk in close agreement with those plotted in Fig. 2.

A laminar flow at the critical radius was obtained finally by mixing glycerol with the milk to increase the bulk viscosity ν to 3.2 cS. The glycerol diluted the milk, but the degree of homogenization measured at a given pressure has been found to be insensitive to a reduction in the oil phase volume of the emulsion when this is low¹⁴; the presence of the glycerol was shown to have no influence on the spectroturbidimetric size analyses.

The combined effects of modifying the valve, reducing Q and increasing ν were calculated to give a value of Re at the entrance

to the recess of approximately 270, decreasing with increasing radius to a value of 100 in the entrance to the valve slit (the critical region). In addition, the flow would be laminar in the central supply conduit and cavitation-free across the valve. The values of d_{vs} obtained at different homogenizing pressures under these conditions are plotted in Fig. 2b. Contrary to any expectation of poorer globule dispersion, it may be seen that values at corresponding pressures agree remarkably well with those achieved with the customary turbulent flow.

These results clearly show that turbulence does not play an important role in the fragmentation process. The possibility that two different mechanisms operate—one appropriate to laminar and one to turbulent flow—to produce identical results is hard to believe.

The degree of homogenization for a given pressure is relatively insensitive to quite large changes in Q , v and the valve geometry; presumably this is a consequence of automatic, compensating changes in valve lift.

Experiment has shown that globules must fragment almost immediately after entering the valve slit. It has been suggested⁷ that, at the slit entrance, globules are drawn into threads before they disintegrate and this seems the most likely possibility. In this region, the flow converges rapidly into the minute crevice and there is a steep velocity gradient. Such a gradient along the line of flow is to be found in a plane hyperbolic flow field and its distorting effect on globules at the slit entrance could be quite similar to the effects that have been observed in the laboratory on fluid drops in that type of field^{15,17}. In those experiments, deformation and extension of a drop in the flow direction occurred; at a sufficiently high shear rate—depending on the fluid system—the drop could be drawn into a cylindrical thread which subsequently disintegrates into several small drops, particularly if the generating flow was abruptly retarded or stopped. In this type of flow threads can be formed even if the ratio of the viscosity of the emulsified drop to that of the suspending medium, η_1/η_0 , is as high as 20 (ref. 15) or, as we have found by experiment, even 50 or more. The high value of 26 in the case of milk would, therefore, seem unlikely to impose any restrictions on the disintegration of molten milk fat globules by this mechanism. By contrast, it seems that in simple laminar shear flow drops do not disintegrate when $\eta_1/\eta_0 > 4$ but merely distort and twist in the flow¹⁶. It follows that, in the experiment above with laminar flow of the milk/glycerol mixture, the fact that η_1/η_0 was 6.4 is incompatible with a simple shear mechanism of break-up; this accords with experiment.

Calculations¹⁷ of the minimum shear rate or velocity gradient G needed to deform a milk fat globule in hyperbolic flow give some idea of the local gradient that would be necessary to do this at the entrance to the valve slit. Thus taking an average globule diameter of 3 μm , a viscosity of 0.01 P for the continuous phase and 5 dyne cm^{-1} for the interfacial tension¹⁸, a value of $2.5 \times 10^6 \text{ s}^{-1}$ is found. For a fairly high pressure drop across the valve the average velocity at the entrance to the slit can be $\sim 10^4 \text{ cm s}^{-1}$; the small upstream approach velocity may be neglected in comparison. Gradients of $G \sim 10^6 \text{ s}^{-1}$ would be possible, therefore, provided that the velocity change occurred over distances of the order of 10^{-2} cm . The rapidly decelerating radial flow of the bulk emulsion across the valve, which succeeds its acceleration into the slit, would then appear to be an appropriate condition for disintegration of the thread-like particles.

The degree of elongation and break-up of a globule probably depends on its precise trajectory into the slit because the velocity gradient across the width of the slit at the entrance would produce a corresponding variation in G over the cross-section. The greatest rupturing effect would be expected in the centre of the stream where the velocity is greatest and G is a maximum, though the length of the disrupting zone may still not favour the greatest break-up effect possible. Thus a single passage of the emulsion through the valve could not produce the maximum dispersive effect; this accords with the fact that

repeated homogenization of the same emulsion at constant pressure progressively increases globule dispersion.

Speculating on the somewhat improved dispersion that can be achieved with breaker rings⁴ and corrugated valve surfaces^{7,19}, it seems likely that this arises from the introduction, by these modifications, of additional regions where accelerated flow can occur.

I thank Professor J. N. Hunt, Applied Mathematics Department, University of Reading, for his interest and Miss S. Higgs and Mrs V. Bines for technical assistance.

L. W. PHIPPS

National Institute for Research in Dairying,
Shinfield,
Reading,
Berkshire

Received May 10; revised September 15, 1971.

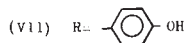
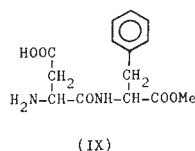
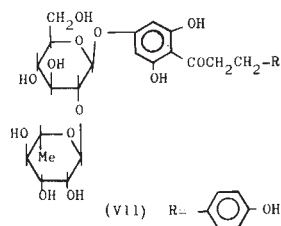
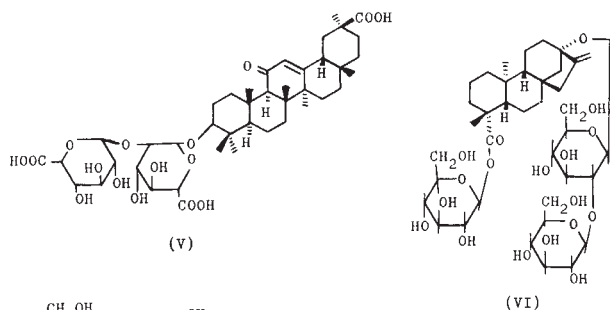
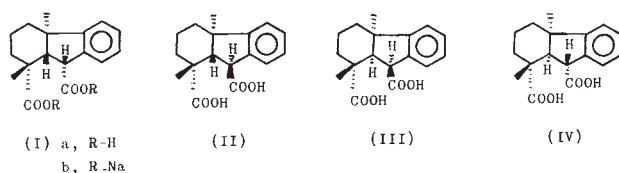
- ¹ Cronshaw, H. B., *Dairy Information* (Dairy Industries Ltd, London, 1947).
- ² Trout, G. M., *Homogenized Milk* (Michigan State College Press, East Lansing, 1950).
- ³ Doan, F. J., *Rep. Pa. Assoc. Dairy Milk Insp.*, **7**, 70 (1931).
- ⁴ Farrall, A. W., *Dairy Engineering* (Wiley, New York, 1942).
- ⁵ Doan, F. J., and Minster, C. H., *Pa. Agr. Sta. Tech. Bull.*, **287** (1933).
- ⁶ Sommer, H. H., *The Theory and Practice of Ice Cream Making*, third ed., 119 (published by the author, Madison, Wisconsin, 1938).
- ⁷ Wittig, A. B., *Mechanics of the Homogenizing Process and the Influence of Emulsion Change Functions on Milk* (edit. by Norsk Meieriteknisk, Forening, 1950).
- ⁸ Loo, C. C., Slatter, W. L., and Powell, R. W., *J. Dairy Sci.*, **33**, 692 (1950).
- ⁹ Loo, C. C., and Carleton, W. M., *J. Dairy Sci.*, **36**, 64 (1953).
- ¹⁰ Rees, L. H., *J. Soc. Dairy Tech.*, **21**, 172 (1968).
- ¹¹ Walstra, P., *Neth. Milk Dairy J.*, **23**, 290 (1969).
- ¹² McKillop, A. A., Dunkley, W. L., Brockmeyer, R. L., and Perry, R. L., *J. Dairy Sci.*, **38**, 273 (1955).
- ¹³ Goulden, J. D. S., and Phipps, L. W., *Proc. Third Intern. Cong. Surface Activity*, C3, 190 (1960).
- ¹⁴ Goulden, J. D. S., and Phipps, L. W., *J. Dairy Res.*, **31**, 195 (1964).
- ¹⁵ Taylor, G. I., *Proc. Roy. Soc.*, **146**, 501 (1934).
- ¹⁶ Karam, H. J., and Bellinger, J. C., *Ind. Eng. Chem.*, **7**, 576 (1968).
- ¹⁷ Rumscheidt, F. D., and Mason, S. G., *J. Colloid Sci.*, **16**, 238 (1961).
- ¹⁸ Horwitz, C., Fremlin, J. H., and Farr, R. F., *Phys. Med. Biol.*, **10**, 385 (1965).
- ¹⁹ Goulden, J. D. S., and Phipps, L. W., *Proc. First Intern. Cong. Food Sci. Tech.*, London (1962).

BIOLOGICAL SCIENCES

New Type of Compound with Strong Sweetness

CHEMISTS have long been interested in sweet substances to be used in place of sugar for medical and dietary purposes, and several such substances have been found in natural sources or as synthetic compounds. Sodium saccharin, sodium cyclohexylsulphamate, glycyrrhizin¹ (V), stevioside² (VI), naringin dihydrochalcone³ (VII), neohesperidine dihydrochalcone³ (VIII), and L-aspartyl-L-phenylalanine methyl ester⁴ (IX) are well known as sweeteners, some of which have been put to practical use. We wish to report a new type of sweet compound with a different structure.

Studies on chemical conversion of pine tree rosin to biogenetically active compounds have been carried out in our laboratory. Recently four stereoisomers (I, II, III, and IV) of 4 β , 10 α -dimethyl-1, 2, 3, 4, 5, 10-hexahydrofluorene-4 α , 6-



dicarboxylic acid have been synthesized from pine tree rosin⁵, one of them (I) had a remarkably sweet taste although the others had no taste. The taste of this compound had some bitterness besides the sweetness (Table I). It is unique in that its structure is that of a simple diterpenoid. Though glycyrrhizin (V) and stevioside (VI) have a similar terpenoid skeleton, they have a glycoside formula as a whole. Correlations between sweetness and structure have been sought by many researchers, and the fact that only one out of four stereoisomers had a sweet taste should make a significant contribution. We are now working on its large scale preparation and on toxicity tests (acute, subacute, and chronic).

Table 1 Comparison of Taste

Compound	Relative sweetness (threshold value for sweetness; conc. % in H ₂ O solution)
Sucrose ⁶	1
(Ia)	1,300~1,800 (0.0006%)
Na salt of I (Ib)	1,600~2,000 (0.0007%)
Sodium saccharin ⁶	200~700
Sodium cyclohexylsulphamate ⁶	30~80
Glycyrrhizin ⁶ (V)	50
Stevioside ⁶ (VI)	300
Naringin dihydrochalcone ⁶ (VII)	300
Neohesperidine dihydrochalcone ⁶ (VIII)	2,000
L-Aspartyl-L-phenylalanine methyl ester ⁶ (IX)	100~200

Compound	Relative bitterness
Caffeine	1
(Ia)	12~18
Na salt of I (Ib)	12~13

We thank Dr Yasuo Abe and the staff of Takeda Chemical Industries Ltd for examining the sweetness and bitterness of compound (I).

AKIRA TAHARA
TADASHI NAKATA
YASUO OHTSUKA

Rikagaku Kenkyusho,
(The Institute of Physical and Chemical Research),
Wako-shi,
Saitama-ken

Received June 7, 1971.

- Beck, K. M., in *Encyclopedia of Chemical Technology*, second ed., 19 (edit. by Mark, H. F., Mcketta, jun., J. J., and Othmer, D. F.), 602 (Wiley, New York, 1969).
- Wood, jun., H. B., Allerton, R., Diehl, H. W., and Fletcher, jun., H. G., *J. Org. Chem.*, **20**, 875 (1955); Vis, E., and Fletcher, jun., H. G., *J. Amer. Chem. Soc.*, **78**, 4709 (1956).
- Horowitz, R. M., and Gentili, B., *J. Agric. Food Chem.*, **17**, 696 (1969).
- Mazur, R. H., Schlatter, J. M., and Goldkamp, A. H., *J. Amer. Chem. Soc.*, **91**, 2684 (1969).
- Tahara, A., and Ohtsuka, Y., *Chem. Pharm. Bull. (Tokyo)*, **18**, 859 (1970).
- Beck, K. M., in *Encyclopedia of Chemical Technology*, 19 (edit. by Mark, H. F., Mcketta, jun., J. J., and Othmer, D. F.), 594 (Wiley, New York, 1969).

Lesions due to Complement in Lipid Membranes

WHEN cells are lysed by antibody and complement (C), uniform bubbles or "holes" revealed by negative staining in the electron microscope appear at the surface. These lesions are probably due to micelle formation in the surface lipid layer (evidence reviewed in ref. 1). C activated by antibody through C8 has also been shown to damage liposomes containing Forssman antigen in their lipid membranes^{2,3}, though not to make holes in them⁴. C activated in the "reactive lysis" system (involving attachment to erythrocyte membranes or other hydrophobic surfaces of an unstable C567 complex which in turn interacts with C8 and C9⁵) causes typical lesions in erythrocyte membranes⁶ and in lecithin liposomes⁷.

Although the detailed mechanism remains to be elucidated, the evidence indicates that the terminal components of C act on a lipid substrate to form micelles and that this is responsible

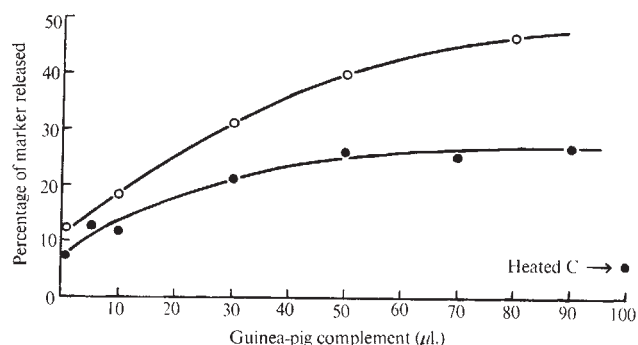


Fig. 1 Release of glucose marker from liposomes (made from extracted sheep erythrocyte lipids), as a function of the amount of guinea-pig C added. 200 μ L total reaction mixture was incubated for 1 h at 37°C. Anti-Forssman antibody dilution 1:10, $\circ$; 1:25, $\bullet$.

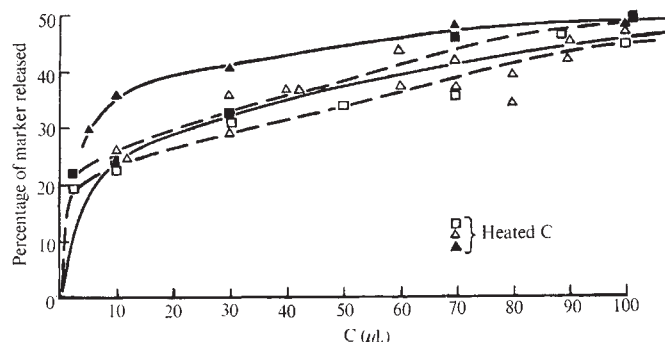


Fig. 2 Glucose marker release with human and rabbit C. Incubation conditions were as in Fig. 1. $\square$, Human C; $\blacksquare$, human C + 1 : 25 antibody; $\triangle$, rabbit C; $\blacktriangle$, rabbit C + 1 : 25 antibody.

for the irreversible cell damage. The only contrary evidence is a report that holes are formed by the action of C5, before cell damage is apparent⁸, and the failure to demonstrate holes in liposomes damaged by C activated by antigen-antibody combination. We wish to report that typical holes are formed when liposomes are damaged by C in both the conventional antibody-mediated and the reactive lysis systems but that no lesions are detectable when C6-deficient rabbit serum is used as the source of C.

lipids⁹. After standing overnight liposomes were separated from most untrapped glucose by passage through a column (1.8 × 50 cm) of 'Sephadex G-25' (coarse), using for elution either the barbitone buffer (pH 7.2) (referred to hereafter as VBS) containing optimal amounts of Mg and Ca described by Mayer¹⁰, or a lower ionic strength buffer (0.06 M NaCl, 0.18 M lactose, 4.87 mM Na barbitone, 1 mM MgCl₂, 0.15 mM CaCl₂ adjusted to pH 7.2). Preparations were stable for at least 3 weeks when stored under N₂ at 3° C. The presence and availability of Forssman antigen were checked by showing that 1 ml. of the liposome preparation would neutralize about 97% of the haemolytic activity in an equal volume of a rabbit anti-Forssman antibody containing 200,000 haemolytic units per ml.

Release of glucose marker was measured by the change in absorbance at 340 nm due to reduction of TPN in the presence of hexokinase, glucose-6-phosphate dehydrogenase and ATP¹¹. Both C and antiserum were dialysed beforehand in the cold against the appropriate buffer to remove pre-existing glucose, and a correction was made for any remaining traces. In most experiments 100 μ l. of liposome suspension was mixed with diluted rabbit antibody followed by varying amounts of human, guinea-pig or rabbit serum as a source of C, and the volume was made up to 200 μ l. with VBS. A suspension of liposomes in buffer was included as a control for untrapped glucose. After 1 h at 37° C the glucose released was measured, and the total glucose present was determined after complete lysis of the liposomes by adding 'Triton X-100' in chloroform at a final concentration of 2%. Release of glucose did not exceed about 5% with Forssman antibody over a range of

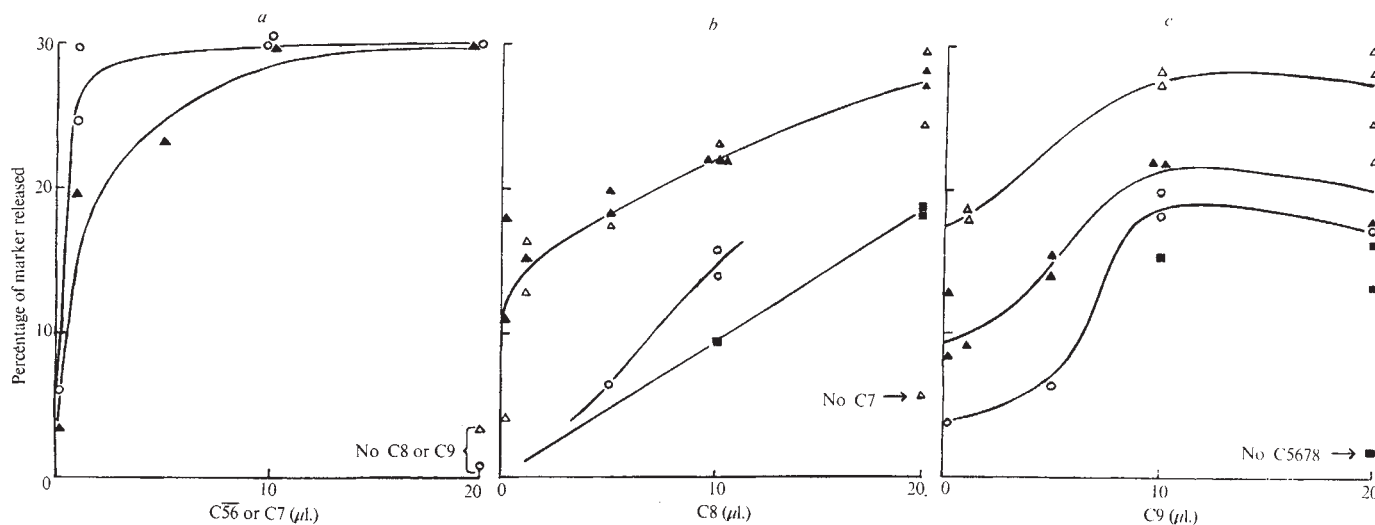


Fig. 3 Marker release from liposomes in the reactive lysis system. Conditions as for Fig. 1. *a*, $\blacktriangle$, 20 μ l. C8 + 20 μ l. C9 + 10 μ l. C7 + variable C56; $\circ$, 20 μ l. C8 + 20 μ l. C9 + 10 μ l. C56 + variable C7. *b*, $\triangle$, 10 μ l. C56 + 1 μ l. C7 + variable C8 + -20 μ l. C9; $\blacktriangle$, 10 μ l. C9; $\circ$, 5 μ l. C9; $\blacksquare$, 1 μ l. C9. *c*, $\triangle$, 10 μ l. C56 + 1 μ l. C7 + variable C9 + -20 μ l. C8; $\blacktriangle$, 10 μ l. C8; $\circ$, 5 μ l. C8; $\blacksquare$, 1 μ l. C8.

Liposomes (aqueous dispersions of vesicles with walls of one or more stable bimolecular lipid layers containing Forssman antigen) were prepared from the lipid mixture extracted from sheep erythrocyte membranes (or whole washed erythrocytes) with 2 : 1 chloroform : methanol, and evaporated to dryness under a stream of dry nitrogen.

Samples of some preparations were dissolved in diethyl ether and added to 0.3 M aqueous glucose, and the ether was removed by evacuation in a rotary evaporator for about 30 min. In other samples dried lipids were allowed to swell in 0.3 M glucose under nitrogen without agitation for at least 2 h. Both methods yielded turbid suspensions containing 5–15 μ mol total lipid per ml. based on the mean composition for sheep erythrocyte

concentrations 10⁻² to 10⁻⁵ with or without heated C (56° C for 60 min) up to a concentration of 1 : 2.5. With antibody and active C, however, as much as 50% of the glucose was released (the amount varied with different liposome preparations, but was reproducible with a given preparation). Fig. 1 shows that the release by different amounts of guinea-pig C in the presence of 1 : 10 or 1 : 25 dilutions of antiserum (approximately 200,000 haemolytic U/ml.) reached plateau values of about 45 and 25% at 1 : 2.5 and 1 : 4 concentrations of C respectively. Fig. 2 shows similar curves for human and normal rabbit C (both of which contained "natural" Forssman antibody), with and without 1 : 25 added rabbit antibody. The lowest concentration of human C tested (1 : 100) caused about half maximal release

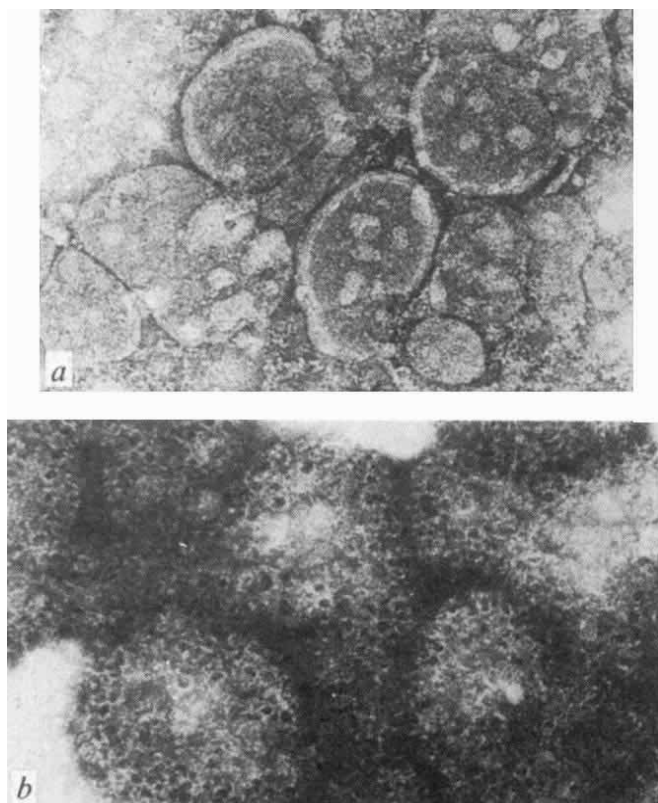


Fig. 4 Electron micrographs (negative staining) of grids coated with liposomes, treated with Forssman antibody and incubated (a) with heat-inactivated human C; (b) with unheated human C ($\times 120,000$).

of glucose, even without additional rabbit antibody, and 1 : 40 rabbit C with added antibody released even more.

When C6 deficient rabbit serum¹² was tested as the source of C for the same range of concentrations no glucose was released in excess of the heated C control. That the C6 deficient serum was not inhibitory was checked by showing that addition of 1 : 10 normal rabbit C produced the expected degree of release even in the presence of three times as much C6 deficient serum.

The effect of different components of the reactive lysis system on liposomes was studied using human reagents prepared as described before^{5,6}. 100 μ l. of liposome suspension was stirred for 2 min at room temperature with a preparation of C56; purified C7 was next added and stirring was continued for 5 min at 37° C. Purified C8 and C9 were added last and the mixture was stirred and incubated at 37° C for 1 h. Fig. 3a shows the effect of varying C56 or C7 in the presence of an excess of the other reagents; glucose release increased as more C56 was added up to 1/10 of the total volume, whereas maximal release occurred with minimal amounts of C7. When the concentration of C567 was constant and the concentrations of C8 and C9 were varied, glucose release was maximal at about 1/10 C9 (Fig. 1b) and 1/10 C8 (Fig. 1c). Considerable release occurred at the highest concentrations of C8 in the absence of added C9, but the inference that C9 is unnecessary for lysis is weakened by uncertainty whether the C8 preparation was completely free of C9. Marker release in the presence of C56, C7, C8 and C9 was increased by up to 7% by 0.01 M EDTA. In the absence of any single component, other than C9, it was minimal.

Attempts to demonstrate typical C lesions on liposomes by negative staining in either system were unsuccessful when carbon coated electron microscope grids were floated on or dipped into the liposome preparation at the end of the incubation period, when damage was expected. Even when the final suspension was prefixed by adding osmium tetroxide, damaged liposomes were not seen. Since it was possible that damaged liposomes might not attach to the grids, we tried allowing the liposomes to adsorb to the grids before damage occurred. Grids coated with 'Parlodion' and carbon were floated at room temperature on the original liposome suspension when using the Forssman antibody system, or on liposomes treated with C56 and C7 for the reactive lysis system. After 10 min the grids with liposomes attached were rinsed in VBS and floated on diluted antibody followed by C or on C8 plus C9 respectively, and incubated at 37° C for 1 h. After incubation grids were washed in VBS (pH 7.2), negatively stained with 2% sodium silicotungstate and dried. Electron micrographs of grids coated with liposomes in this way, and then treated with IgM antibody and heated human C, showed liposome spherules about 0.1 to 1 μ m in diameter covered with small filamentous particles which might be molecules of IgM. The appearance of grids coated similarly with liposomes, but treated with

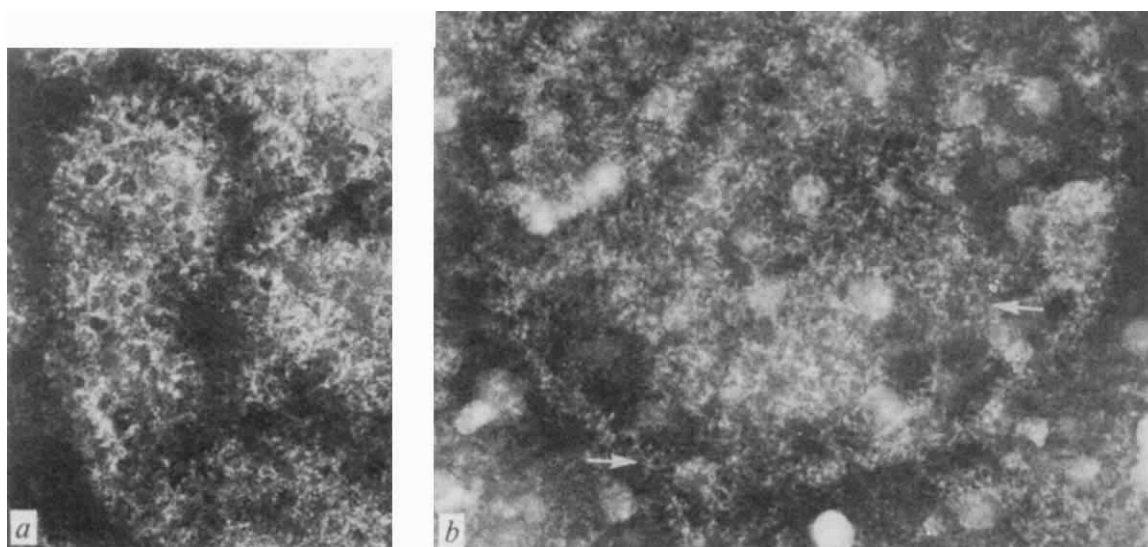


Fig. 5 Liposomes prepared as in Fig. 4, treated with Forssman antibody and a, normal rabbit C; b, C6 deficient rabbit C. Arrows point to ring-like structures ($\times 192,000$).

antibody and unheated human C, differed in that most liposomes had either disappeared or were less prominent, and that both the remaining liposomes and the surrounding surface were covered with typical C holes (Fig. 4). These had a central dark area about 10 nm in diameter, similar to those produced by human C on *E. coli* lipopolysaccharide¹, though the external ring seemed to be narrower. Grids treated with antibody and normal rabbit C had similar holes. With C6 deficient serum "holes" were not evident, but some smaller ring-like structures could be discerned which may correspond to the membrane alterations reported after the action of C1-5⁸ (Fig. 5). Typical lesions were obtained with the complete reactive lysis system, but when C8 and C9 or pretreatment with C567 were omitted none were detected.

These experiments confirm evidence¹ that C-mediated damage to membranes, sufficient to cause them to leak, is correlated with disruption of the membranes by formation of characteristic micellar structures, visualized as holes within them. It is probable that holes were only visualized regularly when liposomes were adsorbed on the grids before disruption because the constituents of the liposomes, which are quite fluid structures, reassemble into smaller fragments when liposomes are disrupted in suspension. No lesions were apparent before the addition of C8 and C9 in the reactive lysis system, nor with C6 deficient serum in the conventional system; therefore the lesions must presumably be the final cause of damage and not incidental byproducts. Since the liposomes were composed only of lipids, the substrate upon which the terminal components of C act is presumably lipid, although it has not been formally excluded that some surface active substance, for example, a peculiar peptide, might be formed from one of the later C components and inserted into the lipid membrane. The report of Lachmann *et al.*⁷ that holes could be produced by the reactive lysis system on pure lecithin liposomes indicates that the substrate is likely to be phospholipid, especially since lipid analysis revealed only a trace of cholesterol in any of the reagents which they used. The extent of any chemical changes may be very small, however, because none have yet been detected either by Inoue and Kinsky¹³ who studied the action of guinea-pig C on liposomes containing Forssman antigen, or by one of us (S. N. P., unpublished) in a system involving human C and *E. coli* cell walls.

T. R. HESKETH
R. R. DOURMASHKIN
SHEILA N. PAYNE
J. H. HUMPHREY
P. J. LACHMANN

National Institute for Medical Research,
Mill Hill, London, NW7, and
Department of Pathology,
University of Cambridge

Received January 28; revised July 5, 1971.

¹¹ Slein, M. W., in *Methods of Enzymatic Analysis* (edit. by Bergmeyer, H. U.), 117 (1963).

¹² Lachmann, P. J., in *Protides of the Biological Fluids* (edit. by Peeters, H.), 17 (Elsevier, Amsterdam, 1970).

¹³ Inoue, K., and Kinsky, S. C., *Biochemistry*, 9, 4767 (1970).

Luminescent System in a Myctophid Fish, *Diaphus elucens* Brauer

The myctophids, or lantern fishes, which migrate diurnally to the surface at dusk, are among the most common and widely distributed deep-sea fishes known. They emit a blue light from tiny dermal photophores¹ arranged in species-specific patterns over the ventral and lateral surfaces of the body. Certain species also possess a large nasal or caudal organ. Although hydrogen peroxide stimulates the photophores to luminesce², nothing is known about the molecular mechanism involved. We report that photophore extracts of the myctophid, *Diaphus elucens* Brauer, cross-react to give light with highly purified luciferin (substrate) and luciferase (enzyme) of the marine ostracod crustacean, *Cypridina hilgendorffii*. The *Cypridina* luminescence reaction is due to the oxidation of luciferin by molecular oxygen, catalysed by luciferase.

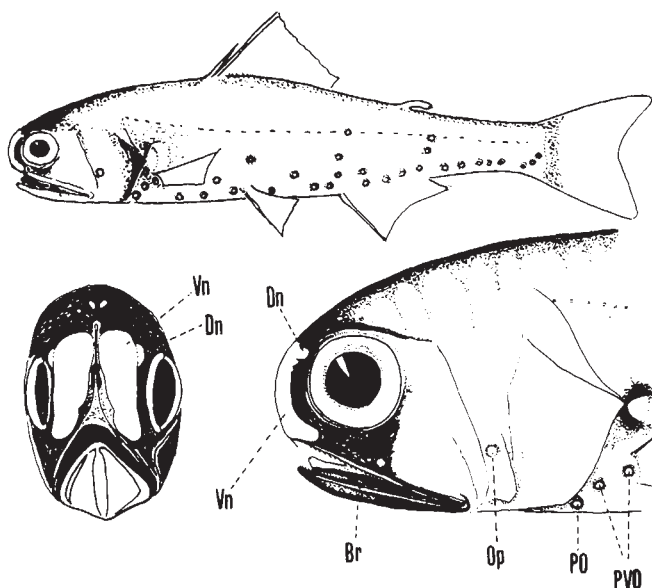


Fig. 1 *Diaphus elucens*, showing luminous organs on trunk and head. Note the large ventro-nasal organ (Vn) and the dorso-nasal organ (Dn). Other luminous organs: branchiostegal (Br), opercular (Op), pectoral (PO), and subpectoral (PVO). Drawn from actual specimen. Terminology of the photophores is according to Bolin³.

Specimens of *D. elucens* were netted at night off Yui, Suruga Bay, Japan, at depths of 20–30 m as they migrated toward the surface from areas of the Bay, 1,000–1,600 m deep. They were preserved at -35° C. Specimens were 102–140 mm long from tip of snout to tail base and 24–31 mm deep. The distribution of the luminous organs is shown in Fig. 1. Cell-free extracts were prepared by grinding 100 dermal photophores in 10 ml. of 0.05 M Tris (pH 8.0) in a glass homogenizer chilled in an ice bath. The homogenate was centrifuged at 12,500g for 10 min at 4° C. The extract was dialysed overnight at 4° C against distilled water. *Cypridina* luciferin (in 0.1 N HCl and stored under argon in an ice bath to prevent autoxidation) and luciferase (1.73×10^{-6} mg/ml., in water) were prepared as previously described^{4,5}. Light intensities

¹ Humphrey, J. H., and Dourmashkin, R. R., *Adv. Immunol.*, 11, 75 (1969).

² Haxby, J. A., Gotze, O., Muller-Eberhard, H. J., and Kinsky, S. C., *Proc. US Nat. Acad. Sci.*, 64, 290 (1969).

³ Kinsky, S. C., Haxby, J. A., Zopf, D. A., Alving, C. R., and Kinsky, C. B., *Biochemistry*, 8, 4149 (1969).

⁴ Knudson, K. C., Bing, D. H., and Kater, L., *J. Immunol.*, 106, 258 (1971).

⁵ Thompson, R. A., and Lachmann, P. J., *J. Exp. Med.*, 131, 629 (1970).

⁶ Lachmann, P. J., and Thompson, R. A., *J. Exp. Med.*, 131, 643 (1970).

⁷ Lachmann, P. J., Munn, E. A., and Weissmann, G., *Immunology*, 19, 983 (1970).

⁸ Polley, M. J., *J. Exp. Med.*, 133, No. 1 (1971).

⁹ Nelson, G., *Biochem. Biophys. Acta*, 144, 221 (1967).

¹⁰ Mayer, M. M., in *Experimental Immunochimistry* second ed. (edit. by Kabat, E. A., and Mayer, M. M.), 148 (Thomas, Springfield, Illinois, 1961).

were measured in arbitrary light units with a MacNichol photomultiplier (RCA 1P21) photometer⁶.

Fig. 2 shows the result of mixing a highly active *Diaphus* extract with *Cypridina* luciferin. The light intensities (directly proportional to reaction rates) are plotted against time according to the equation for a first order reaction. The rate constants were for curve *a*, $4.59 \times 10^{-2} \text{ s}^{-1}$; curve *b*, $2.15 \times 10^{-2} \text{ s}^{-1}$; and curve *c*, $1.99 \times 10^{-2} \text{ s}^{-1}$. They are very nearly proportional to extract concentration (curves *a*, *b*) and independent of luciferin concentration (curves *b*, *c*). The luminescent reaction therefore is first order and resembles the *Cypridina* reaction⁴. The reason for the high initial rate is unknown, but it also occurs in the *Cypridina* reaction⁴.

The emission spectra of the reaction between *Diaphus* extract and *Cypridina* luciferin, and between *Cypridina* luciferin and luciferase, were measured with an Aminco-Bowman spectrophotofluorometer, using a Moseley 7035A (Hewlett-Packard) X-Y recorder. The *Diaphus* extract and *Cypridina* luciferase (+0.7 ml. 0.2 M Tris (pH 8.0)) were each injected into *Cypridina* luciferin diluted with 2 ml. of 0.2 M Tris (pH 8.0). The spectra are virtually identical (Fig. 3) and run from about 400 nm to about 600 nm, with a maximum at around 456 nm and half-intensity width of about 70 nm.

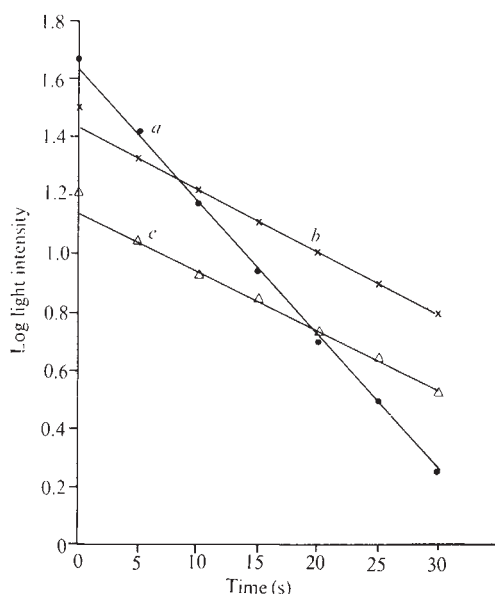


Fig. 2 Luminescence decay curves obtained by injecting *Diaphus* photophore extract into buffered *Cypridina* luciferin. Curve *a*, 1 ml. extract, 0.2 ml. luciferin, 2 ml. of 0.2 M Tris (pH 8.0); curve *b*, 0.5 ml. extract, 0.2 ml. luciferin, 2.5 ml. of 0.2 M Tris (pH 8.0); and curve *c*, 0.5 ml. extract, 0.1 ml. luciferin, 2.6 ml. of 0.2 M Tris (pH 8.0).

Diaphus extract mixed with *Cypridina* luciferin in anaerobic conditions did not luminesce until air was admitted. The activity of the extract was inhibited by immunoglobulin G isolated from rabbit antisera against *Cypridina* luciferase⁷. The inhibition of *Cypridina* luciferase by specific antisera but not by normal sera⁸ indicates that the extract contains an enzyme similar to *Cypridina* luciferase. *Diaphus* extract (undialysed) also gave light with *Cypridina* luciferase. Only a slight stimulation of light emission occurred when *Diaphus* extract was mixed with 0.5% H_2O_2 or 0.5% H_2O_2 with either crystalline beef liver catalase (Worthington, 1 mg/ml., in water) or horseradish peroxidase (Worthington, 0.5 mg/ml., in water). *Cypridina* luciferin mixed with 0.5% H_2O_2 produced a brighter light. Nasal organ (Fig. 1, Vn, Dn) extracts gave results similar to dermal photophore extracts. Light was emitted when muscle and stomach extracts were mixed with *Cypridina*

luciferin and luciferase. Purified luciferin and luciferase (details of which will be reported elsewhere) of the deep-sea decapod shrimp, *Hoplophorus graciliorstris*, also produced light with *Diaphus* extracts and *Cypridina* luciferin and luciferase.

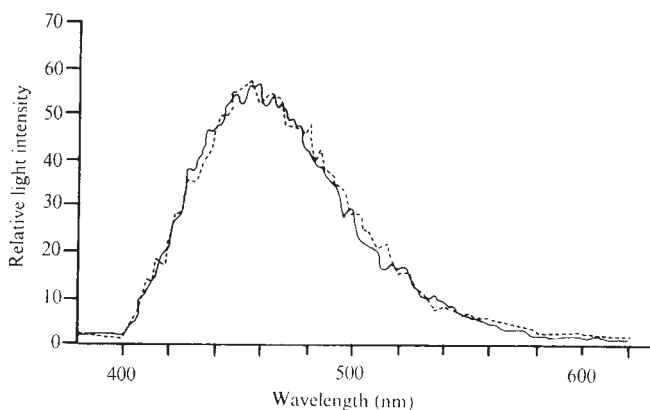


Fig. 3 Emission spectra of *Diaphus* extract-*Cypridina* luciferin and *Cypridina* luciferin-luciferase reactions. *Diaphus* spectrum (—): 1 ml. dialysed *Diaphus* extract, 0.2 ml. *Cypridina* luciferin, 2 ml. of 0.2 M Tris (pH 8.0). *Cypridina* spectrum (---): 0.3 ml. of *Cypridina* luciferase (1.73×10^{-6} mg/ml., in water), 0.2 ml. *Cypridina* luciferin, 2.7 ml. of 0.2 M Tris (pH 8.0). The monochromator was calibrated with a mercury light source but the curves are not corrected for either photomultiplier (RCA 1P21) sensitivity or luminescence decay.

Results with *Diaphus* extracts have been confirmed with extracts of the dermal photophores and infracaudal organ of another myctophid, *Stenobrachius leucopsarus*, collected off the coast of southern California. Cell-free extracts of the ventral photophores of the hatchet fish, *Polyipnus spinosus*, from Suruga Bay, and *Sternoptyx diaphana*, from southern California, also cross-reacted with the *Cypridina* system and showed properties similar to the myctophid system. We conclude that in *D. elucens*, and the other species studied, the biochemical mechanism for luminescence involves a luciferin and luciferase similar to the luciferin and luciferase of *Cypridina*.

We thank Dr B. G. Nafpaktitis and Mr W. T. O'Day for collecting *S. leucopsarus* and *S. diaphana* and Dr T. Abe for identifying *D. elucens*. The study was supported by grants from NSF and the Japan Society for the Promotion of Science under the US-Japan Cooperative Science Program.

FREDERICK I. TSUJI

Department of Biophysics and Microbiology,
University of Pittsburgh, and
Veterans Hospital,
Pittsburgh, Pennsylvania

YATA HANEDA

Yokosuka City Museum,
Yokosuka

Received June 7; revised July 13, 1971.

¹ Nicol, J. A. C., *J. Mar. Biol. Assoc. UK*, **39**, 27 (1960).

² Ancil, M., and Gruchy, C. G., *J. Fish. Res. Board Canada*, **27**, 826 (1970).

³ Bolin, R. L., *Stanford Ichthyol. Bull.*, **1**, 89 (1939).

⁴ Tsuji, F. I., Lynch, R. V., III, and Haneda, Y., *Biol. Bull.*, **139**, 386 (1970).

⁵ Tsuji, F. I., and Sowinski, R., *J. Cell. Comp. Physiol.*, **58**, 125 (1961).

⁶ Chase, A. M., in *Methods of Biochemical Analysis*, III (edit. by Glick, D.), 61 (Interscience Publishers, New York, 1960).

⁷ Tsuji, F. I., Davis, D. L., and Donald, D. H., *J. Immunol.*, **102**, 519 (1969).

⁸ Tsuji, F. I., Davis, D. L., and Sowinski, R., *J. Immunol.*, **84**, 615 (1960).

BOOK REVIEWS

Weakness at Reading

Reading and its Difficulties: a Psychological Study. By M. D. Vernon. Pp. viii+211. (Cambridge University: London, September 1971.) £3.20; \$10.50.

THIS book is a sequel to Professor Vernon's *Backwardness in Reading*, published in 1957, and it deals for the most part with research which has been carried out since that date. A wide variety of experiments are cited: on visual perception, on auditory and linguistic development in children, on the relation of reading to intelligence, and on the social and motivational factors which are associated with high and low scores on reading tests. Finally, Professor Vernon examines some of the evidence relating to specific dyslexia (a disability, or group of disabilities, in which weakness at reading is accompanied by directional confusion, difficulty in spatial and temporal sequencing and very strange spelling mistakes).

As usual, Professor Vernon's standards of thoroughness and scholarship are admirable. The evidence on perception of form and print is particularly well presented; and above all I should like to pay tribute to the chapter on dyslexia, which I wish could be made compulsory reading for everybody concerned with educational planning.

I have one chief criticism. In parts of the book, particularly in the chapters on the relevance to reading of intelligence and of social and motivational factors, Professor Vernon seems to me to have been too charitable to research of dubious merit. Occasionally, it is true, she refers to inadequacies of experimental design or suggests that an author's interpretation of his findings may be questionable. It may well be, however, that educational research, if it is to prosper, requires not mere improvement here and there but radical re-thinking. Countless man-hours have been spent on "measuring" such allegedly useful entities as "IQ" and "reading age", on making judgments about parental support or children's degree of application (sometimes, incidentally, value-laden and arrogant ones, for example, "On the basis of interviews . . . they divided the parents into four classes: demanding, over-anxious, unconcerned and normal", page 107), and on correlating and in some cases factor analysing the resultant scores. Yet an IQ figure is a summary of a person's performance on a wide variety of tasks, the component elements of which are unknown; correlation coefficients are liable to be treacherous even for the sophisticated (because, having obtained

them, we may still not know what to do with them), and surveys of what has happened in a particular educational milieu must surely be of less interest to science than generalizations about what can be made to happen by systematic manipulation of the environment of individual children. I wish, therefore, that Professor Vernon had done more to encourage research workers in the educational field to move in this latter direction rather than content themselves with large-scale surveys of the traditional kind.

This, however, is basically a criticism of current educational research, not of Professor Vernon, who in fact has done an excellent job with the material at her disposal.

T. R. MILES

Medical Illustration

History of Medical Illustration from Antiquity to AD 1600. By Robert Herrlinger. Pp. 178. (Pitman: London, 1971.) £7.

THIS book was designed as the first volume of a two volume series to cover medical illustration from the earliest times to the present day. Regrettably because of the premature death of the author, only the first volume was completed and this covers the period from antiquity to AD 1600. The author, a distinguished medical historian, also received recognition as an art historian and as a result his illustrations are particularly notable for their artistry as well as their appositeness.

There is a masterly foreword by Dr Poynter, director of the Wellcome Institute of the History of Medicine. The book is profusely illustrated with nearly four hundred illustrations, many of which are beautifully reproduced in colour. Apart from the occasional American spelling the translation is good and lively. The text presents a scholarly interpretation and communication of historical research and there is a proper enquiry of the aims of medical illustration at particular periods. Herrlinger demonstrates by text and illustration that schematization is more typical of medical illustrations of the middle ages than naturalism, but everywhere the text shows the erudition and clarity of mind of the author. He regards the early cauterization pictures as examples of the use of indication lines and later points out that couches and beds supporting invalids are symbols of illness rather than actual representations. In like manner, he illuminates the symbolism behind the apparent crudity of many medical illustrations and reaches the conclusion that antique medical

illustration included schematic, semi-schematic and naturalistic forms, in fact, all the categories of modern medical illustration. The early references to "squatting figures" are carried throughout the text and illustrations to make a continuous and recognizable record. Herrlinger explains this curious posture found in many early texts as derived from the need to make thighs and genitals accessible when the corpse is laid on the table for dissection and therefore feels it is more apt to use the term "mensa figures". There are a few spelling mistakes, for example "skillful" for skilful on page 49, but this is unusual and the text is on the whole beautifully printed.

This is a bibliophile's book not only for the quality of the printing and the binding but also because of the many references to title pages, frontispieces and other bibliophilic details to delight the heart of all who love books. It is somewhat disappointing therefore to see that the plates are not always set as conveniently in relation to the text as one would like. With such profuse illustration this can be difficult, but there seems little reason for setting plate 3, referred to on pages 15 and 16, at page 35, and plate 2 is referred to on page 41 and is set at page 18. Again plate 4 is referred to on page 19 and is set at page 36.

There is a list of locations of the manuscripts referred to in the text and an index devoted more to illustrators than illustrations. Herrlinger pays particular attention to Vesalius; nearly thirty pages detail the development and influence of this prince of medical illustrators. Sixteenth century illustrations of surgery and instruments are treated less fully although there is a section on sixteenth century title pages.

These are relatively minor objections, however, to a fascinating history, although I doubt that many medical students would be prepared to spend this amount of money even on a beautifully produced, authoritative and profusely illustrated work of medical history and illustration. Certainly every medical library should have a copy.

EDWARD HITCHCOCK

Training and Testing

Animal Psychophysics: The Design and Conduct of Sensory Experiments. Edited by William C. Stebbins. Pp. xii+433. (Appleton-Century-Crofts: New York, December 1970.) \$18.75.

THIS book is the outcome of a symposium held at the University of Michigan in 1969, during which the

participants discussed their work on the behavioural analysis of sensory function in animals. The book contains eighteen articles, and the emphasis throughout is on the methods by which the animals were trained and tested. Such coherence as the book possesses thus comes from the techniques shared in common by the participants, and not from any of the problems that they are studying by means of these techniques. The book covers a wide range of sensory experiments on animals, including, for example, the detection of ultrasound by bats, of X-rays by rats and monkeys, and of sound by opossums and hedgehogs. In all cases, there are descriptions of how the stimuli were presented and the construction of the apparatus, and details of any idiosyncrasies that make that particular species difficult to work with.

The problems of obtaining accurate behavioural data on the sensory capacities of animals are important; among other things this is the only way in which the significance of physiological experiments on sensation can be assessed. The articles in this book show how well these problems have been solved in a variety of cases, and that it is possible to obtain psychophysical data from animals which are as accurate as those obtained from man. As a whole, however, the book has little unity. The various techniques of animal psychophysics that have been used do not seem to have very much in common with each other, and few general principles emerge. As in other fields of animal behaviour, each species presents different problems which need different solutions, and reading a paper on, for example, how to train and test monkeys helps only a little in designing an experiment for fish. A book this length can also include only an arbitrary and limited selection of the work in this field. The final impression is thus of a fairly random selection of contributions, often interesting in themselves, but only tenuously interrelated.

W. R. A. MUNTZ

Problems Solved

Applied Diffraction Theory. By F. H. Northover. (Modern Analytic and Computational Methods in Science and Mathematics; a Group of Monographs and Advanced Textbooks, No. 28.) Pp. 632. (Elsevier: New York, 1971.) £20.

THIS book provides a mathematical analysis of the solutions to problems involving time harmonic scalar waves and time harmonic electromagnetic waves. The author's selection of obstacles is restricted and the application of his mathematical results to problems in physics and engineering is not always

emphasized. The choice of material is clearly personal and he does not attempt to review the literature relating to diffraction theory. He has, however, succeeded in providing the full mathematical analysis of problems that have interested him for more than twenty years. The mathematical topics are sophisticated and include separation of variables, eigenfunction expansions, special functions, contour integration, asymptotic methods and integral equations.

The early chapters are concerned principally with the propagation of electromagnetic waves around a perfectly conducting sphere embedded in a dielectric medium (a model for very high frequency propagation of radio waves around the Earth surrounded by a nonhomogeneous atmosphere). They reflect the author's interests before 1955. I was astonished and dismayed to read on page 114: "Since 1954 the writer's interest has moved from the subject of tropospheric propagation of radio waves to propagation through ionized gases and laboratory-type microwave diffraction problems. Therefore no attempt is made here to describe work in this field subsequent to 1955."

The next problem to be considered is diffraction of electromagnetic waves by a perfectly conducting cone of finite dimensions. The author published a solution in 1962 and a revised solution in 1965. On reviewing these papers, he found that certain aspects of his earlier solutions were open to criticism. In this book he presents a third investigation, paying special attention to the edge conditions, which were ignored in his earlier papers.

The third and last major topic concerns microwave lenses and the Fabry-Perot microwave interferometer, and is based on the Kirchhoff-Huygens diffraction theory. Fredholm integral equations are used extensively in the solution of some particular problems for interferometers consisting of two curved plates in the form of equal spherical caps.

The introduction to the ideas of diffraction theory in the first chapter is unsound. Insufficient attention is paid to the radiation condition at infinity. The reasoning behind the choice of the velocity potential of the diffracted wave in section 6.1 (the sphere diffraction problem—the acoustic case) is erroneous. There are statements of some possible boundary conditions, but no discussion of these boundary conditions and no mention of impedance boundary conditions. The necessity of imposing edge conditions in certain circumstances receives no attention, even though this is central to the investigation of the finite cone problem in the eighth and ninth chapters.

Also in the first chapter are exact

solutions of some problems involving spherical obstacles. The author obtains some complete solutions using the separation of variables. His solutions by means of the infinite Legendre integral transform method are incomplete and purely formal. It is not clear why the integral transform method is worthy of consideration: the integral representing the solution of even the simplest problem looks intractable and attracts no comment. There is no discussion of the relative merits of the two methods of solution, although an oblique remark appears on page 622.

The third chapter, an account of the WKB approximation, would have been more widely usable (as an introduction) with some worked examples and exercises. The analysis is applied to the Airy equation, but the reader is expected to work through the previous twenty pages of technically difficult mathematics to appreciate the results stated for this particular case. There are three references, the most recent dated 1962. A slightly modified version of the chapter has been published by the author in the *Journal of Mathematical Physics* (10, 715; 1969).

The tenth chapter is devoted to some of the more important properties of linear integral equations. Some knowledge of standard Fredholm theory is presupposed. One reference (to Lovitt's book, first published in 1924) is provided in the sixty-seven pages of analysis written in theorem-proof style. In the author's subsequent use of integral equations (in connexion with the microwave problems) there is only one reference to this chapter. A shortened version of the chapter has appeared in the *Journal of Mathematical Analysis and Applications* (29, 305; 1970).

Much of the detailed analysis is in an appendix (of 142 pages). The mathematical supplement at the end of the book is in two parts, the first devoted to the method of steepest descents and the second devoted to the Watson transformation. This is a useful, well written section. The first part has been published in the *Journal of Mathematical Analysis and Applications* (29, 216; 1970). There are several references in the second part; however, in some cases only authors' names are given.

In my copy, there is a serious omission of the details of references 67–96, and there are two obvious mistakes (on page 18 $r^{-1} e^{ikr}$ appears instead of $r^{-1} e^{-ikr}$ and in section 6.1 of the sixteenth chapter Legendre appears instead of Laguerre).

To summarize, *Applied Diffraction Theory* is a detailed account of the mathematical solutions of a small selection of problems. It will interest a highly specialized readership.

D. PRYS THOMAS

CORRESPONDENCE

Skuas

SIR,—B. R. Johnston's "Skua Numbers and Conservation Problems at Cape Hallett, Antarctica" (*Nature*, 231, 468; 1971) has been read with interest and understanding. However, the report requires further elaboration.

With reference to paragraph 3, it should be said that the resident penguins were moved from the area selected for establishing Hallett Station into other parts of the rookery. They were not killed or shipped from Cape Hallett as the verb "were removed" might suggest. This 1956 operation, supervised by Carl Eklund, USNC IGY, was carried out as humanely as possible, without losses, and in light of information then available on penguin behaviour.

With reference to paragraph 5, the remark that there was no significant loss of monel-metal bands is unclear. The

statement on mean annual loss of breeding skuas, and on the fifty breeding birds lost between 1956 and 1967, requires substantiating data to indicate how these figures were derived. Similarly, figures for chicks fledged/females breeding may be based on end-of-the-season count and may result in greater actual breeding success than really exists.

For the benefit of those unfamiliar with skuas, it should be stated that the species is highly adaptive and quickly takes to feeding on food and other wastes available at all Antarctic camps; and that its willingness to take anything that can be swallowed has contributed to its mortality.

With reference to the last sentence, next to the last paragraph, Cape Hallett is not a Specially Protected Area. A very small part of Seabee Hook inland toward the cliffs has been designated as a Specially Protected Area for terrestrial botanical reasons.

In 1970–71, USARP biologists at Hallett Station began the restitution of the natural area; the work is continuing and will result in returning a large part of the land occupied by Hallett Station to the original inhabitants. The 1971–72 scientific work at Hallett Station includes a skua-banding programme by Dr John R. Baker, Iowa State University, in co-operation with Mr C. J. R. Robertson, Department of Internal Affairs, Wellington, New Zealand, for data which may help to establish the true status of the skua population at Cape Hallett, Antarctica.

Yours faithfully,

GEORGE A. LLANO

*Polar Biology Program,
US Antarctic Research Program,
National Science Foundation,
Washington DC 20550*

British Diary

Monday, November 1

Modern Food and Food Additives (6.30 p.m.) Dr D. W. Kent-Jones, Society of Chemical Industry, in association with the Industrial Division of the Chemical Society, in the Scientific Societies Lecture Theatre, 23 Savile Row, London W1.

Some Aspects of Drying Oils Technology (7 p.m.) Mr G. Hutchinson, Oil and Colour Chemists' Association, at the Queen's Hotel, George Street, Hull.

Tomorrow's World in Telecommunications (7 p.m.) Institution of Electrical Engineers, at the Royal Star Hotel, Maidstone, Kent.

Acrylic Resins (4 p.m.) Mr A. R. H. Tawn, Oil and Colour Chemists' Association, Thames Valley—Student Group, in the Main Lecture Theatre, Slough College, Slough.

Tuesday, November 2

Automatic Circuit Testing (7.30 p.m.) Mr D. Palmer, Institute of Measurement and Control, at the Peahen Hotel, St Albans.

Electrons in Solids (5.15 p.m.) Professor L. Pincherle, University of London, at Bedford College, Regent's Park, London NW1.

Migration and Interaction of Triplet Excitons (5.15 p.m.) Professor H. C. Wolf, Faraday Society, at the School of Chemistry, The University of Newcastle upon Tyne. (Bourke Lecture.)

The Excavations at Kalibangan (5.45 p.m.)

Mr B. K. Thapar, University of London, at the Institute of Archaeology, 31/34 Gordon Square, London WC1.

Wednesday, November 3

Food Additives and Contaminants (3 p.m.) Society for Analytical Chemistry, at the Polytechnic of the South Bank, Borough Road, London SE1.

Henry Armstrong and School Science, 1880–1930 (1 p.m.) Dr W. H. Brock, Royal Institution, History of Science Discussion Group, at 21 Albemarle Street, London W1.

Migration and Interaction of Triplet Excitons (4 p.m.) Professor H. C. Wolf, Faraday Society, in the Department of Chemistry, The University of Manchester.

The Effectiveness of Modern Visual Communication Systems (6 p.m.) Mr B. Stapley, Institution of Electronic and Radio Engineers, at 9 Bedford Square, London WC1.

The Ionosphere and Radio Engineering (7 p.m.) Mr G. Millington, Institution of Electrical Engineers, at the University of Essex, Wivenhoe Park, Colchester, Essex.

Using Existing Resources Better—Planning for Future Growth (11 a.m. symposium) Institution of Chemical Engineers, jointly with the Society of Chemical Industry, at the University of Aston in Birmingham.

Thursday, November 4

Fermentation Pilot Plant Organization and Instrumentation (10.30 a.m., panel discussion) Society of Chemical Industry, Microbiology Group, at 14 Belgrave Square, London SW1.

Hazard Analysis — a Quantitative Approach to Safety (6.30 p.m.) Mr T. A. Kletz, Oil and Colour Chemists' Association, at the Royal Turks Head Hotel, Gret Street, Newcastle upon Tyne.

Migration and Interaction of Triplet Excitons (5 p.m.) Professor H. C. Wolf, Faraday Society, in the Department of Chemistry, The University of Sheffield.

Pressure Transmitter Design Parameters (7.30 p.m.) Mr C. H. Goode, Institute of Measurement and Control, at the Hotel Majestic, Park Place, Cheltenham.

Recent Developments in the Preservation of Foods (6.30 p.m.) Mr S. D. Holdsworth, Society of Chemical Industry, Food Group, at the School of Chemistry, University of Bristol.

Statistical Interpretations (7 p.m.) Mr M. Keegan, Society of Cosmetic Chemists of Great Britain, at the Royal Society of Arts, 6–8 John Adam Street, London WC2.

The Laws of Disorder. Part 1: Entropy; Part 2: The Second Law of Thermodynamics (1 p.m., ICI science films) Royal Institution, at 21 Albemarle Street, London W1.

The Pulsars (5.30 p.m.) Professor F. G. Smith, Institution of Electrical Engineers, Savoy Place, London WC2. (Appleton Lecture.)

Friday, November 5

Computer Controlled Frequency Response Measurements (5.30 p.m.) Mr A. J. Ley and Mr A. J. Martin, Institution of Electrical Engineers, at Savoy Place, London WC2.

Industrial Progress Associated with Analytical Research—Some Personal Experiences (5 p.m.) Mr A. F. Williams, Society for Analytical Chemistry, at the University of Strathclyde, Glasgow.

Making Sense of Music (9 p.m.) Professor H. C. Longuet-Higgins, FRS, Royal Institution, at 21 Albemarle Street, London W1.

Radiolysis of Nucleic Acids and Related Compounds (1 p.m.) Dr G. Scholes, Royal Institution, Photochemistry Discussion Group, at 21 Albemarle Street, London W1.

Saturday, November 6

Inca Architecture of Highland Peru (3.30 p.m.) Miss Anne Kendal, Inner London Education Authority, at the Horniman Museum, London Road, Forest Hill, London SE23.

Reports and Publications

not included in the monthly Books Supplement

Great Britain and Ireland

Science Research Council. The Work of the Rutherford Laboratory in 1970. Edited by J. R. Smith and F. M. Telling. Pp. 206. (Chilton, Didcot, Berks: Rutherford High Energy Laboratory, 1971.) [146]

Papers and Proceedings of a Conference on Research into the Value of Time, held on 7th–8th May 1970, St. Christopher House, Southwark Street, London, SE1. Edited by N. W. Mansfield. (Time Research Note No. 16.) Pp. vi+131. (London: Highway Economics Unit, Department of the Environment, 1971.) *gratis* [146]

PEP Broadsheet 527: Opting out of the NHS—a Review of the Private Sector of Medical Care. By Michael Lee. Pp. iv+28. (London: PEP, 1971.) 50p. [146]

Productivity Measurement—a Symposium for the Seventies. Contributors: R. Allard, J. E. Keal, H. W. Mount, P. J. Samuel, G. R. Smith and A. R. Swannack. Pp. 50. (London: Institute of Personnel Management, 1971.) £1. [146]

Xenobiotica, Vol. 1, No. 1 (January 1971). Published bi-monthly. Annual Subscription price (payable in advance) £15 postage paid. Subscribers in the USA., Canada and Mexico (air-freight) £16; \$39.20 postage paid. Per part £3; \$7.35 plus postage. Pp. 1–104. (London: Taylor and Francis, Ltd., 1971.) [146]

J. J. Thompson Physical Laboratory, University of Reading. Research Report 1970–71. Pp. iv+61. (Reading: J. J. Thompson Physical Laboratory, 1971.) [146]

Prospects in Health. Pp. 24. (London: Office of Health Economics 1971.) 15p. [176]

Agricultural Research Council. Letcombe Laboratory—Annual Report 1970. Pp. ix+42. (Wantage: Letcombe Laboratory, 1971.) 50p. [186]

A Guide to the Performance of the Standardized Rubella Hemagglutination-Inhibition Test. (Based on the publication of October 1970 by US Department of Health, Education and Welfare, Public Health Service, Health Services and Mental Health Administration, Center for Disease Control, Atlanta, Georgia, USA.) Pp. 43+9. Irvine, Ayrshire: Flow Laboratories, Ltd., Victoria Park, Heatherhouse Road, 1971.) 60p. [186]

Potato Marketing Board: Sutton Bridge Experimental Station. Report No. 6: Storage Design and Equipment for Environmental Control in Potato Stores. Part 1: Storage Buildings. By O. J. H. Statham. Pp. ii+30. (London: Potato Marketing Board, 1971.) [216]

Competition in Television. (An Address given by Huw Wheldon, Managing Director, BBC Television, at a Joint Meeting of the Faculty of Royal Designers for Industry and the Royal Society of Arts, 26 April 1971.) Pp. 15. (London: BBC, 1971.) [216]

The Royal Observatory, Edinburgh. Publications—Vol. 7, No. 3: Photographic Photometry with Calcite Filter Calibration. (a) Inherent Error in the Method. By Peter W. J. L. Brand. (b) Its Use in the Near Infrared. By M. T. Brück. Pp. 35–45. Publications—Vol. 7, No. 4: Multicolour Photometry of the Stars in NGC 2264. By K. Nandy. Pp. 47–62. (Edinburgh: The Royal Observatory, 1971.) [216]

Progress in Reaction Kinetics, Vol. 6, Part 1. Pp. 1–74. £1.60; \$4.25. Progress in Reaction Kinetics, Vol. 6, Part 2. Pp. 75–141. £1.50; \$4. (Oxford and New York: Pergamon Press, 1971.) [226]

Daresbury Nuclear Physics Laboratory. Annual Report 1970. Pp. 134. (Daresbury, near Warrington: Science Research Council, Daresbury Nuclear Physics Laboratory, 1971.) [226]

Guide to the Oxford Botanic Gardens. Pp. 42. (Oxford: Clarendon Press; London: Oxford University Press, 1971.) 40p net. [226]

Field Studies Council. The Effects of Industry on the Environment. (Proceedings of a Symposium held at the Orierton Field Centre, Pembroke, South Wales, as part of a Contribution for European Conservation Year.) Pp. 58. (London: Field Studies Council, 9 Devereux Court, WC2, 1970.) [226]

Courtaulds. Report and Accounts, 1970/1971. Pp. 25. (London: Courtaulds, Ltd., 1971.) [226]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 261, No. 839 (17 June 1971): A Discussion on Subcellular and Macromolecular Aspects of Synaptic Transmission. Organized by H. K. F. Blaschko and A. D. Smith. Pp. 273–437+plates 47–90. £7.75; \$20. (London: The Royal Society, 1971.) [236]

A Bulletin of Current Documentation, Vol. 1, No. 1, June 1971. Pp. 1–32. Published June, December and March. Annual subscription 75p for UK; £1; \$2.50 elsewhere. (London: Association of Commonwealth Universities, 1971.) [236]

Cement and Concrete Association. Report for the year 1970. Pp. 55. (London: Cement and Concrete Association, 1971.) [246]

Other Countries

Académie Royale de Belgique. Classe des Sciences. Mémoires. Tome 29, Fasc. 5: Transformations Conformes et Quasi-Conformes des Variétés Riemanniennes Compactes (Démonstration de la Conjoncture de A. Lichnerowicz). Par Jacqueline Lelong-Ferrand. Pp. 44. (Bruxelles: Académie Royale de Belgique, 1971.) 100 francs. [116]

The Use of Modules in College Biology Teaching. Edited by Joan G. Creager and Darrel L. Murray. Pp. viii+173. (Washington, DC: The Commission on Undergraduate Education in the Biological Sciences, 3900 Wisconsin Avenue, N.W., 1971.) *gratis*. [116]

Académie Royale de Belgique. Annuaire pour 1971. Pp. 346+213. (Bruxelles: Académie Royale de Belgique, 1971.) [116]

International Union for Conservation of Nature and Natural Resources. IUCN Publications, New Series, No. 20: IUCN Eleventh Technical Meeting—Papers and Proceedings, New Delhi, India, 25–28 November 1969. Vol. IV, Fifth Session, Commission on Education—Education Workshop. Environmental Conservation Education Among Populations of Rural and Woodland Areas. Pp. 156. (Morges, Switzerland: International Union for Conservation of Nature and Natural Resources, 1971.) [146]

Académie Royale de Belgique. Classe des Sciences. Mémoires, Tome 29, Fasc. 4: Chimie Organostannique—Structure, Mécanisme, Stéréochimie. Par J. Nasielski. Pp. 59. (Bruxelles: Académie Royale de Belgique, 1971.) 160 francs. [146]

Commonwealth of Australia: Department of Supply. Defence Standards Laboratories Annual Report. 1969–1970. Pp. 62. (Maribyrnong, Victoria: Chief Superintendent, Defence Standards Laboratories, 1971.) [146]

Les Sciences Occultes d'Après les Documents Littéraires Italiens du XVI^e Siècle. Par Viviana Paques. (Mémoires de l'Institut d'Ethnologie, No. VI.) Pp. 220. Le Bateau de Berck. Par F. Beaudouin. (Mémoires de l'Institut d'Ethnologie, V.) Pp. 210. (Paris: Université de Paris, Institut d'Ethnologie Musée de l'Homme, 1970 et 1971.) [146]

Australia: CSIRO. Annual Report of the Division of Applied Chemistry 1969/1970. Pp. 41. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1971.) [166]

World Health Organization. Technical Report Series, No. 463: WHO Expert Committee on Biological Standardization—Twenty-third Report. Pp. 94. (Geneva: WHO; London: HMSO, 1971.) 5 Swiss francs; 50p.; \$1.75. [166]

Proceedings of the North American Palaeontological Convention, Field Museum of Natural History, Chicago, September 5–7, 1969. Part G: Ultra Microplankton. 705–1010. Part H: Evolution of Higher Categories. Pp. 1011–1152. Part I: Extraordinary Fossils. Pp. 1153–1272. Part J: Reef Organisms Through Time. Pp. 1273–1482. Part K: Phosphate in Fossils. Pp. 1483–1562. Part L: Cretaceous Biogeography. Pp. 1563–1674. (Lawrence, Kansas: Allen Press, Inc. 1971.) [166]

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 71–21: Massive Ice and Icy Sediments Throughout the Tuktoyaktuk Peninsula, Richards Island, and Nearby Areas, District of Mackenzie. By V. N. Rampton and J. Ross Mackay. Pp. v+16. Paper 70–45: Geology of Ennadai Lake Map-Area, District of Keewatin. By K. E. Eade. Pp. 19. \$1.50. Paper 70–53: Geology, History and Potential of Vancouver Island Coal Deposits. By J. E. Muller and M. E. Atchison. Pp. viii+50. \$2. Paper 70–60: Some Devonian Charophytes from Western Canada. By H. M. A. Rice. Pp. v+23. \$1.50. Paper 70–67: Quaternary Geology Road Logs—Banff Area, Alberta. By Nathaniel W. Rutter. Pp. v+25. \$0.75. (Ottawa: Information Canada, 1971.) [176]

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